

Tidal project worth support

SITTING somewhere in the Department of Energy is a proposal by a team of engineers known as the Severn Barrage Group for a major study of the feasibility of extracting substantial amounts of tidal energy from the flow of water in the Bristol Channel/Severn Estuary region. The outcome of the request for £1.2 million to do 18 months' investigative work will be watched with great interest by all who have a concern that no energy option should be closed out without some pretty sophisticated analysis and without an extended time being available for public debate.

Tide mills are a pretty old concept. T. L. Shaw, writing on the Severn scheme in *Nature*, **249**, 730 (1974), remarks that they can be traced back to 1180, and the idea of harnessing the unusually high tidal range in the Severn Estuary (mean range 7 metres) was first given detailed engineering study as far back as 1933. Even when nature conspires to provide as favourable a site as the Severn Estuary, however, everyone knows that tidal energy suffers from the severe defect that if turbines are simply driven by the flow of water into and out of the estuary, the periodicity of the daily demand for electricity is only spasmodically in phase with the times at which tidal power can make a useful contribution. There are enough thermal generating sets around the country suffering reduced efficiency because of this daily demand cycle for there to be little enthusiasm simply for the addition of another cyclic term, and, what's more, one with a 29-day spring/neap periodicity to bedevil operating conditions even more. It is for these reasons that the French, who have successfully run a 240-MW tidal generator at La Rance, Brittany, for many years, have never proceeded to the larger scheme for which La Rance was the pilot.

The Severn Estuary scheme as now proposed would, however, not be tied to delivering energy in phase with the tides, but would be part of an integrated scheme providing pump-storage facilities for conventional thermal stations. A barrage would close off the estuary between Cardiff and Weston; above it would be the primary basin in which the water level would fluctuate between the present mean sea level and the high spring-tide level—that is, there would be no flooding of land. Just seaward of the barrage, in deep water, would be a secondary basin, maybe 5 kilometres across. Water from this basin could be pumped up into the primary

basin using electric power from thermal power stations. Electricity would be generated both by the fall of seawater at high tide into the (drained) secondary basin and also by the fall of water in the primary basin back into the sea at low tide. Electricity would be generated during the daytime and consumed in pumping at night. In this way a scheme combining tidal energy extraction and pumped storage would take electricity from thermal stations at times at which they might otherwise have to close down.

Rough estimates put the cost at £2,000 million to generate around 4,000 MW during the day (nearly a tenth of the nation's load at present)—in itself rather an expensive investment, though less so when the advantages of the pumped storage scheme are incorporated and consideration is taken of the price, or lack of it, of fuel.

Until now studies on the Severn Barrage have been conducted at a fairly academic level, and only recently has much serious work been done on the obviously major environmental impact; a cursory examination of material published suggests no overwhelming environmental objections. The time looks ripe therefore for a much more extensive feasibility study. Around £1 million has recently been committed to exploratory research for geothermal energy sources which seem less likely to make a major impact on the nation's energy requirements; a similar sum on tidal energy and the effect that the scheme could have on the environment does not look to be out of line.

If a study is funded and finds that the case for some sort of barrage is strong and that environmental objections can be met, there is still bound to be one major question mark hanging over the project. Once into such a scheme there is little benefit gained in calling it off on the way to completion. If costs go awry, if major problems arise, if the energy scene changes dramatically, there is little that a half-completed barrage can be used for. This will doubtless be used as ammunition by those who have a distaste for large technological projects, so it would be more than usually necessary that both the feasibility survey be done exceptionally well, and that the politicians who make the ultimate decision be thoroughly open with the public about the basis of their decision—since they probably won't be around at the time when nerves are being tested. □



Keeping in touch with Soviet colleagues

Miles Reid, Royal Society Visiting Fellow in the Department of Mathematics at Tokyo University and Fellow of Christ's College, Cambridge, writes an open letter to scientists interested in maintaining and improving contacts with their Soviet colleagues

THE growth of international scientific and cultural ties is potentially of enormous value in developing the spirit of cooperation and friendship between nations. I personally believe we scientists can make a contribution towards making this world a safer and a more decent place to live in. But it is also my opinion that Western scientists do not make full use of existing channels of communication, perhaps due to traditional western misinformation as to what is or is not feasible.

Invitations to the West. The majority of Western scientists are probably familiar with the frustrating fact that senior (non-party) Soviet scientists invited to Western conferences find it impossible to take up the invitation.

The first step for any Soviet citizen in obtaining a visa to travel abroad is to get a reference from the Party Committee of his place of work recommending the visit; there are several subsequent steps where hitches may occur, but I believe the first step to be the essential obstacle, since if the *Partkom* is prepared to give its backing the remaining problems may turn out to be soluble.

Now there is some reason to believe that this obstacle may be less serious for younger scientists. This optimistic view is suggested partly by analogy with the case of visits from the USSR to the countries of eastern Europe (a closer analogy than most people in the West will be aware of); and partly by speculation as to the nature of the decision-making procedure within the *Partkom* of universities and institutes.

I would thus like to urge that every possible opportunity be taken to invite the younger generation of Soviet scientists to meetings in the West or to Western universities. In my field of mathematics (algebraic geometry) there is certainly no shortage of excellent young scientists, and visits to the West by such people, even for short periods, should provide for an extremely stimulating flow of ideas and information in both directions.

I would like to suggest that as a matter of form every invitation to a senior scientist should contain a clause to the effect that if the person invited feels unable to take up the invitation, he suggests the name of a junior colleague or research pupil who could be interested in coming in his stead. Of course, one does not necessarily expect

that a research student of a particular scientist would be able to do more than give a token representation, but his presence will allow a flow of ideas, and will do something towards correcting the balance in the make-up of Soviet delegations.

As far as I am aware almost any young Soviet scientist would jump at the opportunity of travelling to the

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West, and be extremely keen to attempt the considerable task of applying for permission. There is probably a practical upper bound of about two months on the length of time a Russian scientist would be allowed out, and one should allow for some uncertainty in the time of arrival.

Contrary to what is commonly supposed in the West, there is no reason of principle why a Soviet citizen having obtained permission to travel abroad should not support himself on the issue of dollars he is entitled to under the currency regulations, although I am informed that university authorities may be very much more impressed by an invitation promising support and even a contribution towards travel expenses.

Even for a (non-party) scientist with an impeccable student and *Komsomol* background the probability of being allowed out is probably no greater than one-tenth, and it seems to be the case that Western organisers who have had repeated failures in attempts to invite Soviet colleagues sometimes get discouraged and give up trying. This is obviously wrong on probabilistic grounds, since if the probability of success is small one should clearly make more attempts rather than fewer. And if one draws a blank after taking reasonable measures over a period of several years (such as writing periodic supporting letters to individual mem-

bers of the university or institute management urging the advantages to both Western and Soviet science to be gained by some particular visit), then one is in a stronger position to complain that the Soviet side is being uncooperative.

There is certainly no way in which invitations addressed to an individual scientist, or letters enquiring as to the best way of inviting either him or one of his students could compromise him or lead to retaliation from the authorities.

I regard suggestions sometimes made that we take a hard-line attitude and boycott delegates we regard as too ‘official’ as potentially extremely harmful and counterproductive. It is my firm conviction that success in this matter will be directly proportional to the amount of patient effort which we are willing to exert.

The copyright convention. In 1973 the Soviet Union signed the Geneva copyright convention, thus bringing to an end the tradition of publishing pirate translations of Western books and journals. The potentially damaging effect of this on Soviet mathematics doesn't seem to have been generally realised. Before the copyright convention any important textbook was published in an edition of 10,000 (say), ensuring that every college, university or technical school was able to obtain a copy for its library. Similarly important Western articles found their way into libraries across the country in the form of translations in *Matematika*. Since the convention the number of textbooks translated has dwindled to an insignificant trickle, and *Matematika* has ceased publication.

Important university or institute libraries get an issue of foreign currency with which they can buy a selection of the essential literature; but this may have the effect of reducing contact with Western scientific ideas down to the scientists at a few major centres. An important additional source of foreign journals costing nothing in dollars is the exchange of publications, and every attempt should be made to extend the existing exchanges. The use of the photocopying machines at the major Soviet libraries remains at present expensive and inconvenient.

Contact by mail. A visitor at a Russian scientific centre is constantly embarrassed by being pumped for information about what's going on in the West; and the plea for preprints, lecture notes and copies of letters is invariably repeated in any discussion. I am not necessarily implying that the Western scientific community has been

mean in this respect in the past, but I'd like to emphasise the desirability of sending as much information as possible.

Although it is true that every item of news received from the West is circulated and discussed with great interest, it is also true that even within Moscow there are many different institutes at which scientists are employed, and there may not be very close contact between them; *a fortiori* this applies to provincial universities. Western scientists need only consider how frequently they make photocopies of preprints or letters for their own use or for passing on to a colleague or student to realise the inconvenience of being deprived of this facility; so it doesn't seem unreasonable to suggest sending a larger number of preprints to a Russian university than one would to the corresponding Western university.

It is perhaps quite striking the extent to which the average scientist depends for his general outlook on informal contact. Thus one might discuss in conversation the work in progress of some colleague and gain thereby an overall impression of some new field of research without having to wait for the precise results to appear as preprints or articles. For a while now I have been trying to apply this principle in written form to try to bridge the gap between Soviet mathematicians in my field and the West, writing with occasional news of new developments (sometimes of which I have only the sketchiest knowledge myself). A slight difficulty is that there are in principle and in practice restrictions on one's correspondents' replies (see below), and one sometimes has to content oneself with the abstract knowledge that the communication is well received, and somehow get over the psychological difficulty of corresponding with someone whose replies are infrequent.

This idea has a potentially invaluable application to an international gathering at which no Soviet delegate is present, and I would like to suggest that if maybe two or three of the participants write their impressions of a few of the most exciting new results, and send three or four copies to interested colleagues in the Soviet Union, then this will do something towards correcting the isolation of Soviet science.

This problem has a converse: Russian scientists also complain that it is very difficult for them to send round copies of their recent work; some of the controversy about Russians not getting fair recognition for their original work perhaps stems from this difficulty.

An article prepared for publication in a Soviet journal will be typed, with three or four carbon copies made, of which several have to be submitted. It would be useful to have some system

whereby a Soviet author who so desires could send out a carbon copy of his typescript to be copied and distributed in the West. This could perhaps be done officially, for example in connection with the translation schemes, or unofficially by private arrangement; those of us who are in correspondence with Soviet scientists should at any rate offer to perform this function.

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There are in principle two legal restrictions on what can be sent out of the Soviet Union. Firstly, the State holds the copyright on anything written by one of its citizens. And secondly an archaic clause of the State Secrets act requires that before sending abroad any written item containing mathematical formulae the sender must obtain the explicit permission of a scientific establishment—which involves the completion of exhaustive formalities, and is usually only possible if the item is already cleared for publication. In practice, however, scientific manuscripts and short items containing formulae are sent abroad rather frequently, as shown for example by the fact that Soviet scientists review regularly for Western journals and translate for the AMS. In general I am impressed by the amount of material that has been permitted in recent years to pass through the mail in both directions, and I would like to interpret this as an indication of the genuine application of one of the clauses of the Helsinki agreement.

It could be argued that the postal regulations, which make it impracticable for papers submitted to Soviet journals to be sent to a reviewer outside the USSR, are partly responsible for the uneven quality of some of the papers appearing in the Soviet scientific press.

Visits to the Soviet Union. The possibilities are as follows: as a tourist; by formal invitation; under the international Cultural Exchanges.

● It is perfectly feasible to visit, say, Moscow or Leningrad as a tourist, and

nowadays the tourist gets a very reasonable deal (travelling with a group may be very much cheaper, and the group activities are not compulsory). When applying for a visa one is required to name the Soviet citizens one intends to meet, but there can be no objection to a scientist who intends to get in touch with colleagues declaring only one name.

It is perhaps worth pointing out in connection with the forthcoming International Congress of Mathematicians that Leningrad is just one night on the train away from Helsinki, and is a town that has considerable attraction to the tourist.

● Soviet scientists may be very willing to attempt to get an invitation made out to a Western colleague to visit them for periods of up to two months. However, it is fair to say that this is not necessarily straightforward. Certainly any Western scientist interested in this possibility should in the first instance write well in advance to a Soviet colleague with whom he has good scientific relations to discuss tactics.

● There are cultural exchanges in operation between the Soviet Union and every Western country. They take place both at the level of visiting fellowships (organised through the Academy of Sciences) and visiting studentships (through the Ministry of Higher Education), and represent almost the only practical possibility for a Western scientist to spend a long period (six months or a year) in contact with a Soviet scientific centre.

I would strongly recommend the latter to any Western research student of recent PhD who is interested in some aspect of Soviet science, is willing to learn Russian, and is prepared to face the difficulties as well as the varied delights of Soviet life. Copies of my report to the British Council containing some of the fruits of my two years' experience in the USSR will be available on request. The very least that Western universities can do to encourage the use of these exchanges is to display the advertising matter relating to them.

In conclusion I would remark that there are many countries in the world where the working conditions of scientists, and the ways in which contacts with the world scientific community can be improved, are subjects which certainly deserve further study. But it seems fair to say that the Soviet Union, the People's Republic of China and Czechoslovakia stand out as the three major countries of the world whose minimal cooperation in maintaining scientific contact amounts to a deliberate affront to the world academic community on the part of their governments. □



Chile is a large, sparsely populated country. Three million of its ten million inhabitants live in Santiago, the capital city, where virtually all economic activity except mining and agriculture is concentrated.

A former Spanish colony, Chile gained her independence at the beginning of the nineteenth century. Subsequently she maintained a remarkable record (indeed a record unique in Latin America and scarcely matched anywhere) for unbroken democracy and liberal government. She was, for example, among the first countries to abolish slavery, and her social services were very well developed for such a poor country. From the 1930s consistent policies by successive governments succeeded in achieving a substantial degree of industrialisation, though mostly with production on a rather small scale and using imported technology.

The country's stability was broken in September 1973 when a military junta overthrew the leftist government of Dr Salvador Allende, who was killed. In the years since the coup the junta has followed a policy of repression and infringement of human rights, and an economic policy which has caused both a large reduction in production with many companies both large and small going bankrupt, and very high unemployment.

Alwyn Eades, who wrote the accompanying article, is at the University of Bristol and worked in Chile from 1967 to 1973. He recently returned there for the first time in three years to attend a scientific congress and work with his former colleagues. He spent two weeks in the Department of Physics of the Engineering School and visited four other institutions. Together the five laboratories represent a very large proportion of the nation's research effort in physics, materials science and related areas. "It is very easy to be misleading about Chile", he says, "and very easy to be misled".

Three years after Allende

Alwyn Eades, recently in Chile, assesses the state of the physical sciences there

DURING the past three years, research in physics in Chile has suffered set-backs from which it will recover only with difficulty. University physics departments have been especially hard hit; they have no protection from the severe budget reductions and loss of staff brought about by the political upheavals. In materials science the position is less bleak and people are hanging on, if only by the skin of their teeth. This is because the centres of applied science have partial independence. The Institute of Materials Testing and Research of the University of Chile, for example, is in a stronger position than the physics departments because it is able to pay (and has to pay) the whole of its research budget from contract work. Only staff salaries are paid by the University.

This reversal is in marked contrast with the previous steady growth of research. Twenty years ago there was little or no research in physics in Chile. Then in the mid-1950s a small number of physicists, trained abroad mainly in England, began to set up research programmes. This effort was initially slow and precarious, but by the late 1960s they had met with such success that the two most important physics departments in the country (both in the University of Chile, one in the Engineering School, the other in the Faculty of Sciences) each had about 40 academic staff including graduate students, nearly all of whom were involved in research.

At this point it began to seem for the first time that there were enough research physicists in Chile to form a stable nucleus for future development, and that some kind of critical mass had been achieved. Evidence for this lay in the establishment of a doctoral programme; until that time Chilean students were obliged to go abroad to get a higher degree. In the early 1970s people felt such confidence in this stability that there was a lot of talk of developing a science policy for Chile in a way which was unthinkable previously. For the first time, Chilean physicists began to feel secure that no longer could an arbitrary administrative decision or the departure of one man have a disastrous effect on the nation's research effort. It was therefore bitterly ironic that the military coup of September 11, 1973 should, almost incidentally to its many brutal acts, set physics research back nearly two decades.

It is worth pointing out here that

the election of Allende did not directly affect science in Chile very significantly. The presidency of Salvador Allende represented much more of a continuation and natural extension of previous government policy in Chile than is usually recognised outside the country. In any case, the principal effects on science research were, initially, considerable governmental encouragement to make research relate to problems relevant to Chile and, later, a reduction in the budget available in hard currency. The latter was a result of economic problems rather than an attempt to restrict science.

The fate of the two physics departments mentioned above since the coup has been quite different. The Department of Physics in the Faculty of Sciences lost most of its staff within a very short time of the coup. Many left voluntarily to seek employment in other countries. Some were imprisoned and/or expelled. In the three years since the coup there has been a bare handful of staff and there has been no research. I was told, for example, that the two main experimental areas, the cyclotron and a low temperature facility for solid state research, have remained closed down. In the past few months, however, new staff members have been contracted and attempts made to restart research. It is surprising to find that those involved seem confident that stable conditions suitable for research may now prevail.

In the other physics department, that of the Engineering School—a right-wing stronghold in a largely left-wing university—the coup had little immediate effect. My own departure two months later was one of the few obvious results. Over the three intervening years, however, the situation has deteriorated very greatly. There have been several waves of sackings for a mixture of political and economic reasons, as a result of which the number of academic staff has dropped from 45 to about 25. Many research groups have completely vanished.

For example, a very impressive semiconductor and microcircuits facility, set up with substantial funds from the Organisation of American States and the Ford Foundation with the support of the Chilean Development Corporation, now lies virtually abandoned. One of these two laboratories is a complete manufacturing facility for microcircuits; the other is a well equipped laboratory for basic research into semiconductors with no one to use it.

This particular state of affairs is still more disconcerting for another reason. Chilean physics has been characterised by too many groups of too few people covering too wide a spectrum of research; moreover, research topics have been chosen because of the training received by staff abroad rather than because of any relevance to Chile. The now-abandoned semiconductor facility was the beginning of an attempt to change this.

The pattern occurs elsewhere. Thus the plasma laboratory, equipped with a well instrumented shock tube and once with six staff members, is now deserted. The only remaining member of the group is completing postgraduate study in London University and is reluctant to return to work alone instead of rejoining an active group with research in progress. Laboratories for Mössbauer spectroscopy and nuclear magnetic resonance also lie idle.

Some groups are not so unfortunate, and in particular X-ray crystallography, biophysics and theoretical physics remain active. The first is particularly important because Oscar Wittke and his team not only do good crystallography but also carry out invaluable service work for industry. This long standing work is unique as an example of collaboration between industry and a physics group in Chile. But even these more fortunate groups are building up problems for the future because of their stagnation and the lack of young people receiving training. They are also handicapped by an arbitrary and inefficient administrative structure and erratic finance. This is not new, of course, but it is an increased handicap when conditions are precarious for other reasons.

Three non-university research laboratories have had mixed fortunes. CIMM is the Centre for Research in Mining and Metallurgy. It is financed by the national Copper Corporation, and since copper mining plays such a dominant role in the Chilean economy, CIMM enjoys a privileged position. In the laboratories the equipment is in use and a large research team is not only providing a service to the mining industry, it is also pursuing a number of exciting original lines of research. I gained the impression that recent events have hardly affected them. One of the very few foreign research workers to enter Chile since the coup, John Burley, an Englishman, is helping to build up the microscopy section.

Two other institutions—INTEC (The Technological Institute) and CESMEC (The Centre for Testing and Quality Control)—have been less fortunate. They were set up by the United Nations Development Programme (UNDP) which provided the basic facilities and

equipment and were subsequently financed by the Chilean Development Corporation (CORFO). In the three years since the coup there has been no direct interference in the running of the laboratories, perhaps because of the quasi-international status deriving from their UN association, but the financial support from CORFO has been cut.

In the case of CESMEC it has been withdrawn completely and they have been forced to become entirely self-supporting from the contract work undertaken for industry. This has necessitated substantial reductions of staffing levels and all projects other than the contract work itself have been abandoned. Moreover, they are functioning entirely on capital, in the sense that they are using the equipment provided by UNDP with little possibility of proper maintenance or purchase of spares and none at all of purchasing new equipment.

The future of both institutions is very precarious. It is possible for contract research organisations in the developed world—Battelle Labs in the USA, for example, or the Fulmer Research Institute in Britain to be successfully self-supporting—but to expect the same in a semi-industrialised country like Chile is a pipe-dream. Nonetheless, both organisations are doing good work and playing an important role in the industrial development of the country. If government financial support is not restored, both organisations may not survive more than a year or two—another reversal of progress in the country which will bring the return to a time when all expertise has to be bought outside the country; even for certain kinds of routine analysis, specimens will once again have to be sent abroad.

CIMM, CESMEC and INTEC were all founded within a decade, which might be a surprise since they now contribute so much to the mining industry which is the mainstay of the economy. However, it is only recently, a century and a half after political independence, that colonial attitudes have faded sufficiently that Chileans have begun to recognise that their own expertise can be as valuable as that purchased overseas.

Repression remains severe in Chile, but in many ways the country is back to normal and on the whole people are not nervous about discussing the situation in an open way. 'Normal' in this context means that there are no longer heavily armed police at every turn, nor military vehicles conspicuous in the streets; despite the continuation of the curfew there is once more a normal, if curtailed, night-life and the public does not in general, show signs of tension or strain. It would be possible for a tourist to get no hint from what he sees of how things are below the surface.

The recent release of prisoners held without trial under the "State of Siege" legislation, however, does not mark the end of repression. Those people who are taken prisoner now are taken in secret and the authorities do not admit to holding them; this is, of course, worse than before. Although I heard of a number of such cases, I am not aware of any scientist who is held prisoner now.

Unemployment in Chile has been extremely high since the coup. Even on official figures it now stands above 15% and is probably higher. But this figure hides enormous variations. An international official, who knew from first-hand experience in voluntary social work, said that in some working class areas unemployment runs at 90%. On



Physics Department and Materials Institute, University of Chile

the other hand there seems to be very little unemployment among the professional classes. The very extensive sackings and cut-backs in all government departments as well as in private industry have reduced employment, but amongst professionals there seems to have been enough mobility and opportunity for them to move overseas.

The ex-Dean of the Engineering School was reported as saying that of 11,000 Chilean engineers, 4,000 are now in Venezuela.

The Chilean Physical Society (SOCHIFI), under its president Claudio Gonzalez, continues to function and hold its scientific meetings in the usual way, although with considerable

difficulty because of the loss of members and reduced financial support. One of its present preoccupations is that, since all elections are prohibited in Chile, it is unable (like many other organisations) to replace those committee members who leave the country or retire. It may shortly find itself with no committee at all. □

USA

Overheads: a growing problem

Colin Norman reports from Washington on a growing dispute involving overhead costs associated with government-funded academic research

THE federal government in the United States will spend about \$3,000 million this year to support research and development in colleges and universities. According to one widely accepted estimate, however, between \$600 and \$700 million of those funds will be taken up by overhead costs such as heating and lighting, university libraries, departmental administration and so on.

The size of those costs has long been a source of irritation between researchers and university officials, and between the universities and the federal government, but there are signs that the irritation may develop into a serious dispute when the budgets of some research agencies are considered by Congress later this year. Moreover, the universities have been expressing considerable concern about a review of overhead costs which has been under way in the Executive branch for the past 18 months. Important principles underlying the support of research in the United States are involved, and university officials are now preparing for battle.

It is easy to see why concern about overhead costs is growing. Overheads—which are usually referred to as indirect costs—have been rising faster than the direct costs of doing research in the past few years, which means that indirect costs have been taking up a steadily increasing share of the federal support for academic research. Since, until very recently, total federal funds for basic research have been declining in purchasing power, university scientists are alarmed that the universities are taking an increasingly large bite out of already scarce resources.

And there seems to be a feeling among conservatives on Capitol Hill that the universities may be using federal research grants as a way to

obtain revenue to support a variety of questionable activities. A staff aide to one right-wing Congressman argued in an interview last week, for example, that "the taxpayer is getting ripped off because the universities are turning basic research into a porkbarrel" for higher education. Indirect costs, he opined, are "a giant subsidy to the universities".

Such sentiments naturally touch a raw nerve among university administrators who are already grappling with steeply rising costs and diminishing federal support. They generally argue, however, that much of the criticism is based on a misunderstanding of the nature of indirect costs and the manner in which they are calculated. Though a number of extremely complex accounting procedure are involved, the basic concept is relatively simple. Procedures differ from one institution to another, but it generally works as follows.

When a researcher applies for a grant for a particular project, he calculates the direct costs of salaries, equipment, technicians' time, computer use and so on. The university then adds a fixed percentage for overheads, and the application is submitted to the federal government. If the grant is awarded, the university keeps the overheads and passes the funds for direct costs to the researcher.

The indirect costs are supposed to cover items and services which are shared by a number of research projects and which cannot be charged to any individual grant. Each university calculates its own indirect cost rate each year, but it is allowed to include only certain items specified by the federal government in a document known as FMC 73-8. The rate charged by the university is subject to audit to ensure that no unallowed costs are included. The rates differ from university to university, with the private universities generally charging the highest overheads and the large state universities, which receive some state subsidies and where economies of scale apply, charging lower rates. At Stanford, for example, the rate is 58%,

which means that for every \$100 required for the direct costs of a research project, the university adds \$58 to the grant application to cover overheads.

A good deal of confusion has arisen because different universities express their indirect cost rates in different ways, with some expressing it as a percentage of researchers' salaries and others as a percentage of total direct costs, but there is one inescapable fact: however they are expressed, indirect cost rates are increasing sharply. According to a Congressional staff investigation published last year, for example, the average overhead cost rate has increased from about 30% in 1970 to about 35% in 1975. At Stanford, the rate has gone from 47% to 58% in the past six years.

The universities can cite good reasons for the increases. Charles V. Kidd, executive secretary of the Association of American Universities, notes, for example, that a number of recent federal regulations, such as equal pay requirements, privacy legislation, occupational safety and health regulations and so on, have placed considerable cost burdens on the universities, and many items, such as fuel costs, have been rising particularly steeply. According to William F. Massey, Vice-Provost of Stanford, for example, the university's fuel bill alone has climbed from \$800,000 to \$5.5 million in four years. Moreover, Kidd argues that because of the general financial squeeze on the universities in the past few years, institutions have been more diligent in claiming their legitimate costs from the government, and that has tended to push the cost rates up.

Nevertheless, in response to concerns over rising indirect costs, the Department of Health, Education and Welfare (HEW), which is the largest federal sponsor of academic research, has been reviewing the ground rules governing indirect cost rates. In July 1975, HEW proposed a number of revisions to FMC 73-8 which would have placed severe restrictions on the types of services which the universities could claim as indirect costs. Under the HEW proposal, for example, the universities would no longer have been able to include the costs of libraries in their in-

direct cost calculations, and some administrative expenses would also have been excluded.

The universities complained bitterly, and last month HEW revised the proposals to make them a little more palatable. The proposed revisions are now under consideration in the Office of Management and Budget, and it will be several months before any formal changes, if any, are made to FMC 73-8. If any revisions are made, they will inevitably restrict the amounts which can be claimed by the universities.

There are indications, however, that some stiff criticisms of indirect research costs may break out on Capitol Hill this year. Some of the Congressmen and Senators who in the past few years have poured scorn on research projects with trivial-sounding titles and who have criticised the National Science Foundation (NSF) for its grant review procedures, are beginning to take a critical look at the increasing indirect cost rates. Thus, for example, Robert

Michel, a Republican from Illinois who was instrumental last year in blocking funds for a behavioural science project which he found distasteful, questioned the rapid escalation of indirect costs in NSF grants when the agency's budget was before Congress last year. He indicated that he would take a closer look at the matter this year. And George Archibald, a key staff assistant to former Representative John Conlan, NSF's chief critic in recent years, is now administrative assistant for Conlan's successor and has already begun to investigate the matter of research overheads for his new boss.

Some of the criticisms are likely to surface when a subcommittee of the House Committee on Science and Technology holds a public hearing on indirect costs on February 22. And the House Appropriations Committee is also expected to probe the matter when the budget for the National Institutes of Health is under consideration.

Few observers here are willing to

predict the outcome of these various executive and legislative investigations, but one veteran science policy watcher, now working for the White House, notes that skirmishes over indirect costs have erupted at various times since the federal government became a major sponsor of academic research in the second World War and he speculated that this skirmish will follow the familiar pattern: there will be considerable argument, the federal ground rules will be tightened and the dispute will die away until the next skirmish arises.

This time, however, there is a potent combination of irritants arising from the steep increase in overhead rates, the serious financial situation of many universities, and a strong desire among fiscal conservatives in Congress to seek ways to cut the federal budget. The dispute goes to the heart of the system by which the federal government supports university research, and it is thus of considerable importance to the overall health of American science. □

Taking politics out of health

KEEPING an earlier pledge to "depoliticise" the National Institutes of Health (NIH), Joseph A. Califano, the new Secretary of Health, Education and Welfare (HEW), last week paid a visit to the NIH campus to announce that the Carter Administration has decided to retain Dr Donald S. Fredrickson as NIH Director. The announcement, delivered to a packed audience of research workers, was included in a gentle pep talk in which Califano again promised to insulate NIH from partisan politics, and gave assurances that he would personally seek more resources for biomedical research.

The announcement, and the fact that Califano went to NIH to deliver it, should help to soothe complaints from biomedical researchers that NIH has been subjected to unwelcome political pressure in recent years. During the Nixon Administration, for example, candidates for NIH advisory committees were screened for their political affiliations, and two NIH directors have recently been fired, reportedly for political reasons. The first, Dr Robert Q. Marston, was dismissed by Mr Nixon in January 1973 for opposing budget cuts and the elimination of some cherished training programmes, and his successor, Dr Robert S. Stone, was fired by Mr Ford in January 1975 for alleged administrative incompetence.

Dr Fredrickson, a well respected scientist and administrator, made known his desire to stay on as NIH

Director, but there was widespread speculation over whether the Carter Administration would retain him or replace him with a political appointee. In announcing the decision to keep Fredrickson as Director of NIH, Califano said that the Administration had looked "only for excellence" and he had not asked any candidate for a post in HEW about his or her political views. Fredrickson, he said, had been selected because he is "the best person to do the job that has to be done".

Although Califano's remarks went down well with his audience, he himself acknowledged that the problems which have been afflicting NIH in recent years cannot be cured simply by ending the political screening of candidates for NIH posts. NIH has been suffering from a combination of budgetary stringencies and rapidly shifting priorities as funds have been channelled into cancer research and, to a lesser extent, heart research at the expense of some other programmes. To a large extent, those shifts in priorities have resulted from legislation which has given the National Cancer Institute a privileged political status in NIH. And there has also been a trend which has resulted in a growing proportion of NIH funds being disbursed as contracts rather than grants—a trend which has increased the central management of biomedical research, but which has met with stiff resistance from academic scientists.

In response to some of the concerns which have been expressed in the past few years about the growing politicisation of NIH and the disparities in funding, Congress established a Presidential commission under the chairmanship of a former medical school dean, Franklin Murphy. The Murphy panel last year issued a number of recommendations designed to reduce the privileged status of the cancer institute and to ease the strains within NIH. The Ford Administration made no move to implement the proposals, however, and the question now is whether or not Califano will act on them.

As for funds for NIH, although they have been increasing in the past few years, they haven't kept pace with inflation. According to an analysis recently completed by NIH's budget office, for example, the proportion of research grants approved by peer-review committees which have actually been funded has declined from 58% to 35% over the past three years, and it will drop to 32% next year if Mr Ford's final budget proposals are implemented. Califano said, however, that "I believe the work you do here, and that basic research generally in health, is critical for our society and needs additional support and resources, and I'll do my best to try and get them for you".

That went down particularly well with his listeners.

Colin Norman

SEVESO

Seven months on

Alastair Hay updates developments since the Seveso disaster in northern Italy last year

SEVEN months after the explosion in the trichlorophenol reactor at Seveso which resulted in the release of tetrachlorodibenzo-*p*-dioxin (dioxin), more details of its consequences are becoming apparent. The large number of organisations involved in assessing damage and formulating policy for decontamination of the area has prevented a crash programme. There has, however, been considerable agreement as to a general plan of action—a 'clean-up' operation to try to remedy damage, the full extent of which is still unknown.

According to Mr Alex Rice of Cremer and Warner—the British firm of consulting chemical engineers acting as 'technical auditors' of the decontamination programme—the work of removing dioxin

"is proceeding, and there will be a high degree of reclamation of property and land in a reasonable period of time. The nature of the discharge of the reactor contents resulted in a differential degree of dioxin contamination in different areas. It has been necessary, therefore, to arrive at a graded response to solve the problem of decontamination.

At the moment, in the most severely contaminated area designated as 'Zone A', hard surfaces are being cleaned and it is hoped that some 80% of the residents evacuated from the area will be able to return to their homes in a few months. Meanwhile, in 'Zone B', less severely affected by dioxin and where inhabitants have been able to remain in their homes, vegetation in certain selected areas is being cut down and incinerated".

From the evidence available at Seveso, Cremer & Warner estimate that 2.5–3 kg of dioxin was released in the reactor discharge. On this basis and through information obtained from a model simulating the explosive reaction at the ICMESA chemical plant, they suggest that only a few hundred grams of dioxin was actually deposited on vegetation and other surfaces. They maintain that the majority of dioxin was too finely dispersed and in a particulate form too small to permit precipitation to occur, with the result that thermal air currents carried the material high into the atmosphere. Conditions on the day of the explosion were ideal for the photochemical decomposition of dioxin at these altitudes, but there is no evidence to substantiate that such a degradative process actually occurred.

Earlier reports suggested that the

dioxin was percolating through the soil at Seveso more quickly than recorded on previous occasions in different localities. More recent investigations, however, confirm that 98% of the dioxin is strongly adsorbed on the top 4cm of soil. Some of the methods of soil sampling in the earlier stages of the decontamination programme were not rigorous enough to exclude contamination of lower layers by material on the soil surface. The later investigations confirm previous reports in the literature that dioxin is relatively immobile in soil.

The question of the teratogenic properties of dioxin is still causing considerable anxiety to women in Seveso. The Lombardy regional government ruled last August that pregnant women from the contaminated zones around Seveso could have legal abortions, but local doctors have in many instances consistently refused to terminate pregnancies, and only a small proportion of pregnant women from Seveso have had abortions in Italy. The fetuses from these women are currently being examined for any signs of abnormalities and the results of the investigations should be available by March.

The reluctance of the Italian doctors to perform abortions has forced many Seveso women to seek a termination in other countries. It is doubtful whether any of the fetuses from these abortions will be examined for malformations. Doctors in Britain have voiced their concern over this loss of potentially valuable information on human teratology. They argue that there should be some agreed international code of practice on the matter to prevent it recurring and add that in Britain in particular there are arrangements to facilitate abortions and examination of the aborted fetuses for signs of abnormalities.

In the next few months women from Seveso who were in the first trimester of pregnancy in July last year will reach full term. The children born to them and to the mothers who conceived after the explosion are those most at risk from the teratogenic properties of dioxin. It is proving an agonising time for these mothers as no one can reassure them that their 'dioxin children', as they are known locally, will be unaffected. For them, as for all the people of Seveso, certain reassurances are impossible; it is just a matter of waiting.

● A report in the Turin paper *La Stampa* this week says that many children at a school in the Seveso area are suffering from skin rashes.

IN BRIEF

European energy policy moves

Several important aspects of European energy policy were discussed in talks in London last week between Mr Anthony Wedgwood Benn, the UK Energy Minister, and his West German and Dutch counterparts. The meeting was part of a series Mr Benn is having as chairman of the EEC Council of Energy Ministers.

The ministers agreed that an early decision was needed on the question of increasing the uranium enrichment capacity of Urenco, the consortium established by the three countries which is facing problems because of Dutch concern about Germany's controversial nuclear deal with Brazil. The ministers also tacitly confirmed that the European Community fusion project, JET, was far from dead by agreeing that an early decision on its siting was needed.

No official comment was available from the UK Department of Energy on an appeal last week from the EEC Energy and Research Commissioner, Guido Brunner, who urged Britain to drop its opposition to a Euratom loans plan and its insistence on a minimum safeguard price for oil. These issues could become politically connected with the JET issue.

Brunner also appealed to the USA and Canada to maintain nuclear fuel supplies to the Community, and detailed the Community's efforts in reactor safety and nuclear export controls. A Community team of experts is shortly to visit Washington to discuss these matters.

UK mercury report

"Reassuring" is the word coming from the UK Department of the Environment (DoE) on the environmental impact of mercury in Britain. A report released last week (*Environmental Mercury and Man*, HMSO, £1.40) is the department's tenth pollution paper, and the product of an interdepartmental working group on heavy metals.

The average person in the UK, it says, is "at no discernible risk" from exposure to mercury, but for environmental and health reasons a comprehensive knowledge of the concentration and distribution of mercury must be developed and the toxicology of mercury understood. The report says more research is needed on both the conversion of mercury to and from methylmercury compounds and the environmental pathways of mercury across the water-sediment interface in the aquatic environment; research on the mechanisms of its toxic action and on low level exposure to it over long periods is also necessary.

Help for computer industry

UK government plans to concentrate on electronics as part of its drive to boost exports were revealed last week. The proposal, part of the Labour government's overall 'industrial strategy', followed an announcement that the National Enterprise Board (NEB) is to become involved in the computer industry through a newly-created NEB subsidiary, INSAC Data Systems. The idea initially is to stem fragmentation in the software sector, and later to become involved in minicomputers and peripherals. The news coincides with the publication of details of a consultant's report on the European computer industry which forecasts a bleak outlook for microelectronics in the absence of a radical restructuring of the industry.

New genetics draft

The consultative document drawn up by the UK Health and Safety Commission (HSC) last August containing draft regulations for the control of genetic manipulation experiments in Britain is thought to be still under discussion with the principal scientific interests concerned.

A report appeared last week stating that the HSC's controversial all-embracing clause, requiring notification from any person carrying on "any activity intended to alter, or likely to alter" the genetic constitution of any microorganism, has now been rewritten. The new notification clause which, it was said, the HSC intended to put in its place, reads:

"No person shall carry on any activity which, by using biochemical manipulation

of extra-cellular nucleic acids, is intended or likely

(a) to insert genetic information into organisms, and
(b) to circumvent the natural barriers to such insertions, and
(c) to propagate that information, unless notice has been given . . ."

This proposal, which went out before Christmas, was a new draft for comment, and does not necessarily represent the HSC's final proposed definition of genetic manipulation experiments.

Mediterranean discussions

Representatives from fifteen of the 18 nations surrounding the Mediterranean met last week in Split, Yugoslavia, to discuss proposals for combating pollution of the sea they all share. They meet again in Athens this week.

THE production of food is bound to overshadow many other human activities increasingly in years to come. Scientific agriculture is currently beset with many problems and would-be interveners. A trend in recent years has been to take away the authority of agriculturists over the methods used in agricultural production. The excuse seems to be that those who till the soil cannot be trusted either to 'protect the environment' or to produce food that is sufficiently free from 'contamination' to satisfy the ideals of consumerists.

An inevitable result of these bureaucratic machinations is an increase in the cost of producing food. The advent of a new administration in Washington has given rise to gleeful anticipation of more power by those who oppose many of the technological practices used in agriculture. Such opposition will increase the cost of food production further.

The success of scientific agriculture has made groceries so cheap and plentiful in the USA that the food supply is taken for granted. This may be attributable to the fact that food is so easily obtained that it has lost its aura of being a treasured gift of beneficent Mother Nature. The urban population, now two generations removed from farm life, knows very little about food production. As a result, consumerists, defined as individuals who are quite certain of what is best for the interests of consumerists, hold sway increasingly over consumers, who are apparently somewhat bewildered by it all.

For example, the successful campaign to cast out Red Dye No. 4 from maraschino cherries was based upon the finding that the dye produced

tumours in laboratory rodents, and a grateful populace should have thrown the deadly preserved fruit into the garbage. However, we learn that in the supermarkets there was a run on red maraschino cherries by

Agriculture 1977



THOMAS H. JUKES

hoarders who feared an impending ban.

Consumerists seem not to realise or acknowledge the basic facts of agriculture and nutrition. Some of these are that food contains substances that are toxic at high levels but are sufficiently harmless in the amounts actually present that they offer no finite hazard to health. Such substances include pesticide residues as well as other adventitious contaminants. Food can be produced without such residues, or it may in some instances be refined to remove them, as in the case of sugar.

But such finickiness, carried out to quieten the apprehensions of an opulent minority, do not fit the pattern of making an effort towards achieving abundance and low cost of food in the face of a rapidly-increasing requirement by a burgeoning global population. A few months ago, there was an uproar by consumerists over the FDA tolerances for 'filth' in foods. This was a great source of amusement to anyone who had worked on a farm, and who knew, as a fact of life, that grain and rats are constant companions. Indeed, some accident of design has made rat faeces the size and shape of wheat kernels, and hence the two items are virtually inseparable by the usual screening processes. Canned vegetables and tomatoes have always contained insect parts, more so in pre-pesticide days.

A misapprehension in some quarters is that food of plant origin is somehow 'purer', 'closer to Mother Earth', and 'more healthy' than food derived from animal products. Actually, toxicants, including oestrogens, goitrogens, alkaloids, and fungal carcinogens, occur naturally far more often in plant foods than in animals. Domestic animals, serving as sources of meat, milk and eggs, can serve as 'filters' to metabolise and remove such substances, or to reduce their levels in the human diet. Cereal grains, of course, will continue to be the prime source of calories for human beings throughout the world. The business of feeding people must go on apace, and, to this end, let us hope that the 'Washington consumerist crowd' will not be given the opportunity to pour any more gravel in the gears that they introduced in 1976.

correspondence

Nuclear pros and cons

SIR,—As one of the large group of Nobel prize winners having a strong aversion to nuclear energy and its consequence but no “technological background in fields relevant to nuclear technology,” I would like to express agreement with a portion of the article by Jan M. Döderlein¹ entitled “Nuclear power, public interest and the professional,” but strong disagreement with much of the rest.

His point about uninformed sponsors of statements was also emphasised in 1973 by the late Eugene Rabinowitch, a person concerning whose judgment and expertise in the area of science and public policy no one could quibble: “If, at a certain point, their conclusions begin to be affected by extra-scientific reasons, they must have sufficient intellectual honesty to state: ‘Up to this point, I spoke as a scientist; from here on I will speak also as a politically, ethically or ideologically committed citizen . . .’” Still, anyone but the most naive petition signer must understand that sponsors of socio-scientific news releases are, and always will be, selected on the basis of the fact that the general public tends to be influenced by lists of the names of ‘dignitaries’ of one kind or another (perhaps I should add “so far”).

One particular paragraph in Dr Döderlein’s essay seemed to me to be especially noteworthy:

Probably no technological decision in the history of mankind has been the subject of so many detailed studies, so much open discussion and such broad public participation. Amongst it all, some nuclear critics have given prominence to ethical aspects of the nuclear decision. This is justifiable to a certain extent since the application of nuclear power—indeed of any power—raises ethical questions. To carry this view to extremes in the way it is sometimes done, however, represents a vain attempt to set aside fundamental laws of nature and society.

One of the hitherto inevitable consequences of the “laws of nature and society” has been war. Given enough time, conviction and willing hands, and enough spears or rocks, the human species could certainly be doggedly annihilated. However, the existence of nuclear weapons, and the proliferating capacity to make more, makes ultimate mass slaughter not only possible but probable—I know of no technical fixes for human madness and misjudgment. Surely this statement requires no know-

ledge of the technology of nuclear power. The ethical and social consequences of the impending plutonium economy are at the very core of the problem^{2,3}. We cannot rely, in a world of easy overkill, on “political decisions made in the face of uncertainties”. The “uncertainties” associated with nuclear power, both of the electric and military variety, are unfortunately of much larger potential danger than those we have faced in our previous history.

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¹ Döderlein, J. M., *Nature*, **264**, 202 (1976).

² Scientists’ Declaration on Nuclear Power, issued by Union of Concerned Scientists (1208 Massachusetts Avenue, Cambridge, Mass. 02138; August 1976).

³ *The Plutonium Economy: A Statement of Concern*, issued by National Council of Churches of Christ in the USA (475 Riverside Drive, New York, New York 10027; September 1975).

Evolution and growth

SIR,—One can agree with Professor Tuzo Wilson (January 20, page 196) that, on the Earth, economic growth cannot continue for ever. But can we plan for stability? It is just not true to say that “undisturbed Nature is stable”. Evolution has proceeded as a result of instability. Ecosystems approach stability when they are in a steady state, but even in the steady state phase they are not in fact stable, but oscillating between quantifiable limits. Sudden increases in the size of both populations and of the area they occupy are characteristics of species in an active state of evolution; these are phases of multiplication during which selection pressure is reduced. They alternate with phases of division, in which population size and area of colonisation shrink, and during which selection pressure is increased. As a result, the phyletic line splits, isolation occurs, and new species may arise. Perhaps humanity will be moving into such a phase in the immediate future—but not necessarily so. For evolution in Man is not only controlled by biological processes. Civilisations evolve as a result of psycho-social controls. Under such conditions natural selection no longer operates within a gene-pool, but within an “idea-pool” and it is competition between ideas that leads to evolutionary progress.

Wilson may well be right when he claims that it is the exploitation of energy that has resulted in the sudden

increase in the size of the human population. Certainly we can argue that traditional energy supplies must become scarcer in the future. But psycho-social evolution does not necessarily proceed in phase with biological evolution. If our technological advance continues, it may well solve the energy problem. However, it must do more than that if there is to be any further growth of population. We must also solve the problem of living space and the problem of material resources.

On the Earth there is a limit to growth imposed by the fact that both living space and resources are finite. No such limits are imposed in outer space. The only possibility of continued growth would seem to depend on space colonisation. G. K. O’Neill’s space colonies (*Physics Today*, September 1974) could in their first generation exploit solar energy and lunar and asteroidal materials. They could overcome all the constraints that prevent growth on Earth.

So maybe the future of Man will lead to a bifurcation. Some of us will go into space and multiply; the rest of us will stay on the Earth, and divide.

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Journal abbreviations

SIR,—The abbreviation of periodical titles is a chore to authors and editors, and their secretaries. Abbreviations are also a source of confusion to readers, whom one hopes outnumber the authors. The saving in type-setting which is achieved by the use of abbreviations probably does not justify all this effort.

The British Standards Institution has published a word-abbreviation list (BS 4148: Part 2: 1975) but, like the *World List of Scientific Periodicals*, this does not always provide unique or unambiguous abbreviations. The French *Industrie minérale*, for example, shares the abbreviation *Ind min* with the British *Industrial minerals*; *J math phys* stands equally for *Journal of mathematical physics* or *Journal of mathematics and physics*.

I suggest that the practice of abbreviating titles should be discontinued, in favour of full and accurate citations.

Yours faithfully,

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news and views

Mars: questions and answers from Viking

from Geoffrey Eglinton and James R. Maxwell

THE Viking mission has been a remarkable success. So much so that NASA recently announced the start of an extended mission whereby the four spacecraft will continue to gather data on and around Mars for the next one and a half years to see out a complete Martian year of 687 Sols, each of 24.6 hours. But what of the all-important question: "Is there life on Mars?" NASA's official view of the results of the life detection experiments is that "... they have been puzzling. They neither prove nor disprove the existence of life on Mars". Indeed, the soil is surprisingly "active" but the question is whether this activity is chemical, biological, or possibly both. In fact, most of the researchers involved claim that none of the responses observed necessitates a biological explanation. Appropriately reactive species can be invoked in terms of a surface chemistry controlled by interaction with the atmosphere and with ultraviolet light from the Sun. Thus, understanding the properties of the Martian soils seems to be the province of physicists, chemists and geologists rather than biologists.

It is difficult not to wax lyrical about the Viking mission, with so many 'firsts' for planetary exploration. The mission has simultaneously supported four sophisticated interactive spacecraft at an interplanetary distance. The landing sites were chosen by teams only after study of radar surveys made from Earth and of detailed photography from orbit. In effect, the teams have used the orbiters as their eyes and have directed them to inspect likely sites.

The two spacecraft arrived at Mars last summer. Viking I went into orbit on June 20 and released its lander on July 20, while Viking II arrived on August 7 and sent in its lander on September 3. Pictures and data continued to flood into the Jet Propulsion Laboratory at Pasadena, California until late November when Mars passed out of effective radio contact behind the Sun. The main findings to date

have been published in NASA SP-408 and in three issues of *Science* (August 27, October 1 and December 17, 1976). On December 16 the orbiters were signalled to resume photo coverage and the landers to start up their operations once again. The 18-month extended mission will provide surface and orbital photographic coverage, temperature and water vapour observations of the surface and atmosphere, seismic monitoring and additional analyses of soil and rock, including further life detection experiments. The landers may experience the onset of a planet-wide dust storm when Mars nears perihelion this spring. As part of the new phase of exploration, Viking orbiter II's orbit was changed on December 20 to give a closer approach of 800 km and a higher inclination orbit of 80°. As winter approaches in the northern hemisphere, the north polar cap should grow and the south polar cap recede, while the orbiters keep a detailed watch on changes in surface features, temperatures and water vapour concentrations. Some time this month, Viking I's orbiter will skim by the Martian moon, Phobos, and radio back pictures and thermal measurements taken from only 48 km (30 miles) distance. Rocks less than a metre across should be visible and an explanation found for the mysterious lineations apparent in the photographs transmitted last September. It is likely that the lineations—layers, flow lines, impact or volcanic crater chains...?—hold the key to the origin of this small moon and its partner, Deimos.

The biological experiments are continuing on both landers with fresh soil being tested in each case. These experiments seem unlikely, however, to do little more than harden up the picture already obtained, that is, rapid release of oxygen after contact of the soil with water vapour, absorption and evolution of carbon dioxide, the oxidation of organic nutrients to carbon dioxide, and the synthesis of small

amounts of organic matter from carbon monoxide/dioxide mixtures. These positive results contrast with the molecular analysis of the surface dust by pyrolysis-gas chromatography-mass spectrometry which show that it is virtually free of all pyrolysable carbon compounds. For example, no nitriles, which are pyrolytic products of amino acids were observed at detection limits of ~ 1 part in 10^9 . One possibility that has been suggested is that there is a minute population of microorganisms not detected by the pyrolysis technique and which is unresponsive to the biological tests. It is more likely that the 'biology' can be treated in terms of photochemical weathering and surface chemistry.

The basic need now is to know more about the geochemical circumstances at the surface of Mars. These are clearly very different from those on Earth and a valid interpretation of results from life detection experiments needs an understanding of this chemical environment and of the processes involved in the synthesis and destruction of organic material at the Martian surface. The considerable quantities of carbon which must have been present in the early Martian surface and atmosphere and have arrived in meteorites remain to be accounted for. Certainly some is in the atmosphere and, possibly, mixed in the polar caps as CO_2 and a little CO; some carbonates may also be present in the surface materials. Presumably the processes of surface weathering, which occur in an environment dominated by ultraviolet, low temperatures and the absence of liquid water, are such that any organic compounds formed are rapidly oxidised. These processes would also erase any organic geochemical evidence of past life.

The recent paper by Oyama in *Nature* (265, 110; 1977) highlights the problems facing chemists and biologists. To explain the evolution of oxygen and the evolution and absorption of

carbon dioxide in the Gas Exchange Experiment, Oyama proposes that nascent oxygen from ultraviolet irradiation of CO_2 diffuses to react with alkali and alkaline earth metal oxides, there, by generating peroxides and superoxides, which would be capable of evolving oxygen in the presence of water vapour. Alkaline sites would then be produced which could absorb carbon dioxide. Evolution of carbon dioxide is explained in terms of the presence of "coats" of sulphuric acid on mineral surfaces. However this scheme seems tortuous and premature when so little is known about the composition of the surface. Other hypotheses are appearing but this open-ended situation necessitates patient and detailed dovetailing of the Viking data with results drawn from laboratory

simulation studies.

All of these considerations emphasise the tantalising nature of the Viking mission. The experiments on board are sophisticated in that they have revealed the complexity of the Martian surface-atmosphere relationship but they do not allow the more detailed studies which are now necessary to unravel the surface chemistry involved. Here a returned sample mission, as with the Apollo project, contrasts with the Viking type of remote analysis mission. In the case of the returned sample, new data lead to fresh hypotheses and models which can then be tested interactively, thus generating fresh data and fresh questions. With the Viking mission, the amount of testing that can be done to check on hypotheses or models is limited, though remarkable.

Fitting Flixborough into a pattern

from Henry Chilver

THE explosion of a chemical processing plant at Flixborough on June 1, 1974 was one of the most serious industrial disasters of recent times in this country. More than two years after the disaster, it is very interesting to read D. E. Newland's fascinating account (*Proc. R. Soc. Lond. A351*, 525; 1976) of the mechanics of the temporary pipework, the failure of which was the cause of the disaster. Catastrophes such as Flixborough raise important questions: are engineering skills adequate to build safe, complex engineering plant? What controls are introduced to ensure safe designs? Questions of these sorts raise complex issues, involving the safety of people and the supervision of engineering design and construction. All engineering disasters must be explored as thoroughly as possible and—it is hoped—understood. Each disaster has its own detailed explanation, in the case of Flixborough an explanation in terms of the structural mechanics of the temporary pipework. But how does the Flixborough disaster, seen as the failure of a vital structural component, fit into the general pattern of failures in engineering structures?

Before we attempt to answer this question it is important to try to identify some general pattern of engineering failures as a whole. A general pattern is certainly discernible in the field of construction engineering, where failures of large structures in particular are very spectacular. The first point to emphasise is that major disasters are of course rare events. This is a tribute to the quality of design and construction skills, which are generally of a high order; nevertheless, it is still important to learn, through the analysis of disaster, how we can further im-

prove design, construction and operation.

Structural failures are of three main types, and may involve mixes of these:

- (1) Unknown structural or material deficiencies; for example, the material used may be sub-standard or a vital component may be accidentally damaged.
- (2) Unknown forces in the environment of the structure; for example, a long-span bridge may suffer exceptionally high winds, or a building may suffer unusually severe earthquakes.
- (3) Unknown forms of behaviour of the structure within its environment; for example, a new form of oscilla-

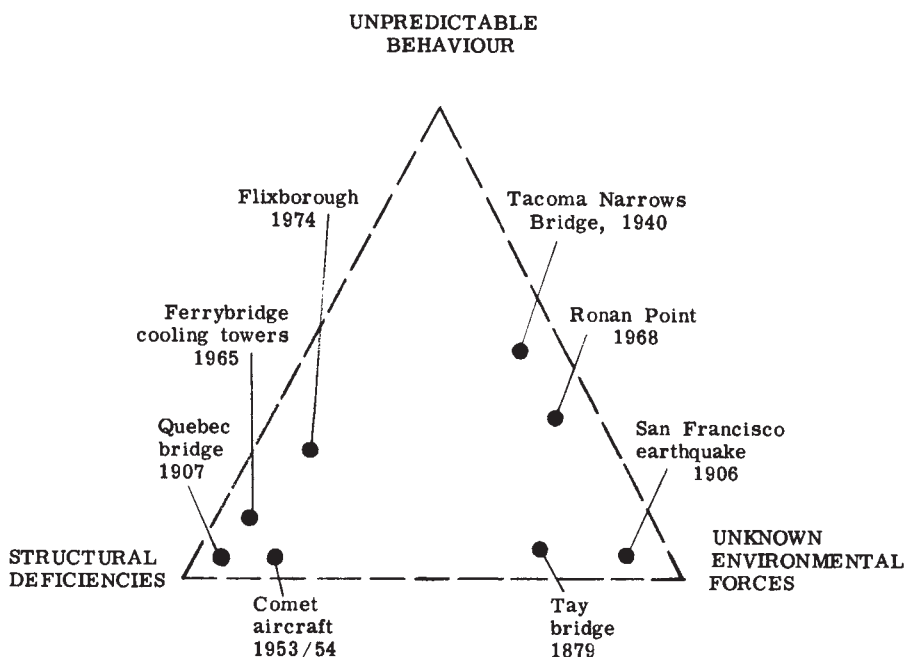
tion or buckling may occur for a new structural form.

Within the framework of these three main types of failure (and these concepts can be extended to other areas of engineering) we can construct a 'triangle' of failures, the corners of the triangle being the three main forms. Within this triangle we can locate failures of different sorts; where a failure involves 'mixes' of different types we can indicate the position appropriately within the triangle.

One of the early disasters of structural engineering was the collapse of the Tay railway bridge in 1879 (Sir Alfred Pugsley, *The safety of structures*, Arnold, 1966; this very interesting book gives more details of some of the failures described briefly in this note). In very high winds, this multi-span bridge across the Tay collapsed while a mail train was crossing; 75 people lost their lives. Although there may have been some design faults, the predominant cause was a lack of knowledge of the intensity of the actual wind forces on such a structure. This early disaster lies, therefore, towards the corner of "unknown environmental forces".

The San Francisco earthquake of 1906 is famous for many reasons, not least for the advances made, after this experience, in the design of structures against earthquake forces. But structural failures during the earthquake were predominantly due again to a lack of understanding of earthquake forces.

Taking again an example from the field of bridges, a famous accident was the Quebec bridge disaster of 1907, when a large cantilever bridge collapsed while under construction into the St Lawrence River with the loss of 75 workmen. This was caused by defec-



tive design of a member of the frame of the bridge. Good design would have shown this to be defective, and hence a basic structural deficiency was built into the structure. This disaster has a position towards the corner of "structural deficiencies".

In 1940, the suspension bridge, newly-built to span the Tacoma Narrows, near Seattle, in the US State of Washington, collapsed after violent oscillations of its deck and towers, luckily with no loss of life. Surprisingly, the failure occurred in what appeared to be only a moderate wind of about 40 mph. The cause of collapse was an inadequate understanding of the interaction of the structure with its environment, leading to large oscillations of the bridge. The resulting behaviour was unpredictable at the time, but became better understood subsequently, after research studies.

In 1953 and 1954, three accidents occurred to Comet civil airliners—all involving serious loss of life. These were caused by structural failure of the pressure cabin brought about by fatigue. The root of the trouble appeared to be cracking near one or other of the windows of the pressurised cabin. In this sense, the structure again had an in-built structural deficiency.

In 1965, a group of cooling towers at the electricity generating station at Ferrybridge in Yorkshire was blown down in a moderate gale, although they had apparently been designed to withstand high wind pressures. This failure is probably in the class of structural deficiency because the thin shells used had severe vertical cracking which probably lowered their stability.

In 1968, some multi-storey flats collapsed at Ronan Point in London after what appeared to be an explosion on one floor. The cause of collapse was traced to the explosion of leaking gas on one storey; the explosion caused structural failure on that floor which in turn led to further structural failures on other floors, rather like the collapse of a row of dominos. Although the gas explosion was unpredictable, there is a case for designing structures in such a way that collapse of one structural component does not lead to general catastrophic failure. This example lies predominantly near the corner of "unknown environmental forces".

How does Flixborough fit into this general pattern? The cause of collapse, as Newland now explains with fascinating detail and exactness, was due to the squirming and jack-knifing of some temporary pipework. The cause of failure was an inbuilt structural deficiency, not implied in the original design, in the form of the temporary pipework.

Can we make any general deductions

about structural failures in the light of this very simple analysis? We begin by looking more generally at the three corners of the 'triangle' of failure. In any engineering design and construction, not all the environmental forces likely to be encountered are known, although designers hope to take account of all the relevant forces. These forces can only be found from experience and research. We must always accept some risk (although we hope this is very small) of encountering a hitherto unknown environmental force of serious magnitude. As design and construction progress, we may have to accept some unfortunate disasters towards the corner of 'unknown environmental forces' in the triangle. At the corner of 'structural deficiencies' it should be possible to eliminate most disasters by thorough supervision of design and construction, particularly if conventional materials are being used.

Where, as in the top corner of the triangle, an 'unpredictable behaviour' emerges, it is difficult to blame engineers for failures. In this area engineers have the responsibility, clearly, of ensuring that research is encouraged in directions in which more knowledge of behaviour is needed, but the risk of a hitherto unknown form of failure emerging should not be discounted.

The risk of structural failure is, in reality, very low. Failures frequently make headline news, and correctly so if lives are lost or put at risk. As engineering design and construction progress, so our experience of structures, of their environments and of their behaviour also evolves. We can only progress by combining experience with research into relevant problems. This is a field in which the scientific study of the natural phenomena involved has a vital role. □

Early assemblage of Carboniferous amphibians

from Andrew Milner

FOR those who are interested in the evolution of terrestriality and the early radiation of terrestrial vertebrates in the Palaeozoic, the fossil record remains frustrating. Although amphibians were present and diversifying in the Upper Devonian, it is not until well into the Upper Carboniferous that amphibians and reptiles are comprehensively represented by rich and diverse assemblages.

The five stages of the European Carboniferous are the Tournaisian, Viséan, Namurian, Westphalian and Stephanian and the majority of Carboniferous tetrapods known to us are of Westphalian age. The major Westphalian assemblages are the lake assemblage from Newsham, Northumberland, the pond assemblages from Linton in Ohio, Nýfany in Czechoslovakia and Jarrow in Eire, and the terrestrial assemblages from Joggins and Florence in Nova Scotia where the terrestrial tetrapods are found as small individuals trapped inside the hollow stumps of giant lycopods. The amphibians and reptiles from these and other contemporary localities are currently referred to about 30 families, most of which have no ancestors or close sister-groups among the known earlier tetrapods. Our concept of the coal-swamp fauna is based on the Westphalian tetrapod fauna which is of great inherent interest but is too late to be directly relevant to the fish-amphibian transition or the earliest

adaptive radiation of tetrapods.

The tetrapod faunas of the Tournaisian, Viséan and Namurian are represented by a small number of specimens or restricted lake assemblages from West Virginia, Nova Scotia, Scotland and Germany. These are being restudied by several workers, in particular Panchen, Carroll, Andrews and Beaumont. Not merely are the early Carboniferous amphibians few in number but they also tell us little about the origin of later groups. Panchen (*Palaeontology* **16**, 179; 1973) has shown that *Crassigyrinus* from the Scottish Viséan, is an ichthyostegid-like relict. More recently he has studied *Pholidogaster* and *Otocratia* and found them to be colosteids with a referred specimen being an anthracosaur skull (Panchen *Phil. Trans. R. Soc.* **B269**, 581; 1975). The Viséan amphibians from West Virginia described by Romer and Hotton have proved to be all colosteids or anthracosaurs. The other major groups of this age, the loxomatids and adelogyrinids are currently being redescribed by Beaumont and Andrews respectively. But though the loxomatids, colosteids and anthracosaurs may each have a few known descendants, mostly confamilial with them, neither they nor such contemporaries as they have, shed light on the origin of most later tetrapods.

As a result of the work described above, the situation has been developing where all the early Carboniferous

material which could be studied, was being studied, without any significant new material being discovered. The Namurian gephyrostegid from the Ruhr described by Boy and Bandel (*Paläontographica* **A145**, 39; 1973) was a significant find, in a new area but, being a terrestrial erratic in a marine horizon, it is unlikely to be the precursor of further discoveries.

This then, is the significance of the discovery of a Namurian assemblage of fish and amphibians from Cowdenbeath announced by Andrews *et al.* in this issue of *Nature* (page 529). It has already produced as many specimens of amphibian as were previously known from the early Carboniferous of Europe and most of the bone-bed has yet to be prepared. It is still difficult to assess the diversity of the fauna for though some skeletal material is articulated, most is fragmentary and will take time to identify. So far, it appears to be a more extensive version of the Viséan assemblage from the Gilmerton Ironstone. If it is like other large assemblages from the Carboniferous, a careful search may reveal the small and rare components of the fauna such as the occasional terrestrial reptiles now known as accidental occurrences from Linton and Nýřany (Carroll & Baird *Bull. Mus. Comp. Zool. Harv.* **143**, 5; 1972; Reisz *J. Paleont.* **49**, 522; 1975). The significance of the find is three-fold. First, it offers the possibility of finding earlier, more primitive representatives of the many families of tetrapods which currently appear so suddenly in the Westphalian. Second, it will permit new morphological studies of these early tetrapods. The preservation is good and the specimens are not severely crushed. Specimens such as the nearly complete *anthracosaurus* will yield much information. Third, the assemblage may provide some palaeo-ecological information about these animals. Most Carboniferous amphibian material was collected in the last century from restricted exposures and is now only available in museum collections, having been through the filter of morphologically orientated collectors. One cannot be certain what was collected and what was missed. This new find was systematically collected by gridded squares and offers the possibility of a quantitative ecological study of a Carboniferous lake fauna.

The Cowdenbeath fauna may only take us back one step from the Westphalian, but it is a long awaited step. However the most relevant amphibians to the water-land transition remain the Upper Devonian ichthyostegids from East Greenland. Although they were collected nearly fifty years ago, little progress can be reported in our understanding of them as much of

their anatomy remains undescribed and no first-hand study of them has been published for twenty-five years. □

Bleak outlook for superheavy nuclei

from P. E. Hodgson

THE announcement by Gentry *et al.* (*Phys. Rev. Lett.* **37**, 11; 1976) of the detection in the mineral monazite of the superheavy nucleus with charge 126, and possibly of other superheavy nuclei as well, has stimulated a series of experiments to confirm this startling discovery, and also many theoretical calculations of the stability of such nuclei. Several of these have been published very recently: the experiments provide no evidence for superheavy nuclei, and the calculations make it very unlikely that they are stable enough to be observed.

The first experiment, by Bosch *et al.* in Germany (*Phys. Rev. Lett.* **37**, 1515; 1976) is very similar to that of Gentry *et al.* They irradiated small grains of monazite with 2 MeV protons, and examined the spectrum of the emitted X rays. They found no sign of a peak in the region where one would be expected if the superheavy nuclei had been present in the same proportion as claimed for the sample studied by Gentry *et al.* They go on to suggest that the peak observed by Gentry *et al.* is really due to the gamma decay of the first excited state of ^{140}Pr , produced in their sample by protons on traces of ^{140}Ce . This contaminant peak should have been removed along with the background due to other contaminants, but Bosch *et al.* claim that the background subtraction was not done correctly.

The second experiment used quite a different technique. Stephan and his colleagues from France and Poland (*Phys. Rev. Lett.* **37**, 1534; 1976) have made a mass spectrographic analysis of about one gram of a sample of monazite from the same place as the original sample. After the mass separation, the ions were collected on a quartz plate, and this was subsequently irradiated for one week in a nuclear reactor. Superheavy nuclei are expected to undergo fission when hit by a neutron, and this is very likely in the high neutron intensities to be found there. The fission fragments would make tracks in the quartz plate which would be visible under the microscope after etching. Careful examination of the quartz plate showed no fission tracks in the region where they would be expected from superheavy nuclei.

The original measurements of Gentry

et al. were made on small inclusions of monazite in mica at the centre of the so called 'giant haloes'. The quantity of superheavy nuclei that they claimed to have found corresponds to about 10^{-4} g of monazite. The mass spectrographic analysis sets an upper limit to the amount of superheavy nuclei in monazite of 10^{-12} g per g. Thus to maintain the original result one would have to say that the concentration of superheavy nuclei in the giant halo inclusions is about 10^8 that of normal monazite, which seems very unlikely.

C. Holbrow of the California Institute of Technology has now studied haloes in detail and finds that some of the naturally-occurring haloes could be produced by ^{244}Pu (this issue of *Nature*, page 504). Although there is very little of this isotope remaining now because of its relatively short half life of 82.8 Myr, there could have been enough of it present when the mineral was originally formed for it to make haloes before it decayed. Giant haloes could also be produced by the alpha particles that are sometimes emitted during the spontaneous fission of ^{244}Pu . The X rays observed after proton bombardment of the inclusions could also come from Te fission fragments and not from superheavy nuclei. This work therefore provides an alternative explanation of the giant haloes that does not involve superheavy nuclei. Confirmation of this possibility requires mass spectrographic identification of the very small amounts of ^{244}Pu that still remain, or of its decay product ^{236}U , or of the fission fragments of ^{244}Pu .

The theoretical calculations of the stability of the superheavy nuclei of $Z=126$ are mainly concerned with the half lives for fission and for alpha and beta decay. For the nuclei to be detectable on naturally-occurring minerals the shortest of these half lives must be comparable to the estimated age of the Solar System, or about 10^9 years. If any of the half lives were shorter, the nuclei would have all decayed long ago.

Some very thorough calculations have recently been published by Möller and Nix (*Phys. Rev. Lett.* **37**, 1461; 1976) following earlier calculations by Wong (*Phys. Rev. Lett.* **37**, 644; 1976). The first calculated the single-particle energies in a spherical one-body potential and found that there is a well-marked neutron magic number at 228, but no proton magic number at 126. There is proton shell closure at 124, so on this evidence alone the nucleus with $N=228$, $Z=124$ is the best candidate.

The more detailed calculations of stability were made using the droplet model of Myers and Swiatecki (*Ann. Phys.* **84**, 186; 1974) with microscopic corrections evaluated by the Strutinsky method (Brack *et al. Rev. mod. Phys.*

44, 320; 1972). Essentially this method gives a soundly-based theoretical expression for the nuclear binding energies, and the fine adjustment of its parameters is made so that it gives the correct results for the heaviest known nuclei.

Brack *et al.* find that the nucleus with $Z=126$ and $N=228$ is stable against beta decay but has a half life against spontaneous fission of only 39 ms and an alpha-decay half life of only 18 yr. These half lives are extremely sensitive to the calculated binding energies, so that there are uncertainties in these figures of several powers of ten, but even allowing for this it seems that the nucleus is far too unstable to have survived. Major changes in the theories used in these calculations would be required to give sufficiently long half lives.

It thus seems at present that the evidence for superheavy nuclei has been seriously weakened. □

Viroids are covalently closed circular RNA

from Roger Hull

VIROIDS are one of the enigmas of plant virology. They are pathogens of higher plants and appear to exist only as self-replicating uncoated ribonucleic acid molecules with many unusual properties.

Much of the knowledge of these properties is based on studies on two viroids, the causal agents of potato spindle tuber (PSTV) and citrus exocortis (CEV) diseases. Using gel electrophoresis and electron microscopy, T. O. Diener and coworkers reported the molecular weight of PSTV to be about 80,000–90,000 (*Virology* **53**, 70, 359; 1973); J. S. Semancik and coworkers determined a molecular weight of 125,000 for CEV (*Virology* **53**, 448; 1973). These molecular weights are much smaller than those of the RNAs of plant viruses. Thermal melting curves, resistance to nucleases and gel electrophoretic behaviour show that viroids are compact highly structured molecules with a considerable amount of base pairing which makes it difficult to obtain reliable molecular weight estimates using gel electrophoresis. Several models for the molecular structure have been proposed, the most recent suggesting that they are single stranded RNA, base-paired to form hairpin-like shapes (*Virology* **55**, 70; 1973; *Virology* **63**, 160; 1975).

In a recent paper (*Proc. natn. Acad. Sci. U.S.A.* **73**, 3852; 1976) H. L. Sanger *et al.* describe the results of experiments which lead to a different

interpretation of the structure of viroids. They confirmed that viroids were single-stranded molecules by showing that native undenatured nucleic acids had similar molecular weights to denatured molecules. Using sedimentation equilibrium analysis they obtained molecular weight values of 127,000 for PSTV, 119,000 for CEV (from two different hosts) and 110,000 for the viroid causing cucumber pale fruit disease. This confirms the previous estimates of the small size of viroid RNA and shows that they have genetic information to code for a protein no larger than 10,000 daltons. In fact protein synthesis experiments (*Virology* **61**, 486; 1974) indicate that viroid RNA is not translated *in vitro*.

Sanger *et al.* went on to determine the structure of viroids. Thermal denaturation showed that each of the viroids they studied had considerable base pairing and that there was one relatively long double helical region and not short hairpin sections as in tRNA. Electron microscopy of native nucleic acid revealed that all three viroids have rod-shaped or dumb-bell shaped molecules, as had been previously reported for PSTV (*Virology* **55**, 70; 1973). When viroids which had been treated under strongly denaturing conditions were examined in the electron microscope a variety of structures ranging from rod-like molecules (renatured during the preparation process) to single-stranded circles were found. Sanger and his colleagues then attempted to analyse the nucleotides at the 5'-end and 3'-ends of the viroid molecules. Using standard techniques (phosphorylation of the 5'-end with 5'-polynucleotide kinase and ^3H -labelling of oxidised 3'-end and capped 5'-end by reduction with ^3H -borohydride) they were unable to detect either 3'- or 5'-nucleotides. From these and other observations (resistance of viroids to snake venom phosphodiesterase) they concluded that viroids are single-stranded, covalently closed circular RNA molecules.

So far in nature, circular molecules have been found for double-stranded and single-stranded DNA and have been suggested for some double-stranded RNA replicative intermediates (for example, *Virology* **70**, 360; 1976). Viroids therefore appear to be the first examples of a new class of molecule. Sanger *et al.* point out that they differ from other naturally occurring covalently closed circular molecules in that they are much smaller and that the base pairing is not between complementary strands but within one strand. This structure would also explain why viroid RNA is not translated using *in vitro* systems.

The observations of Sanger *et al.* should help in the current investiga-

tions in several laboratories into the origin, mechanisms of replication and pathogenicity of viroids. One major question is whether this new class of molecule occurs normally in uninfected plants; if so, how do they become pathogenic? It is already thought that DNA might be involved at some stage in viroid replication (*Virology* **64**, 106; 1975; *Nature* **256**, 753; 1975); in fact there are sequence homologies between PSTV RNA and DNA from both infected and healthy plants (*Proc. natn. Acad. Sci. U.S.A.* **73**, 2453; 1975). The search is now on to find circular single-stranded RNA and DNA molecules of viroid size in both infected and healthy plants. I feel that the search should not be restricted to plants. It will be interesting to know how widespread is the occurrence of single-stranded circular RNA molecules and whether they are involved in diseases of organisms other than higher plants. □

Most leptons come in pairs

from W. T. Toner

THERE was a precursor to the discovery of the ψ : muons and electrons were found in such large numbers in proton-nucleus collisions that a new interaction or particle had to be invoked to account for them. (Boymond *et al. Phys. Rev. Lett.* **33**, 112; 1974; Appel *et al. ibid.*, 722; *News and Views* **254**, 103; 1975). Many new studies were begun whose results continue to accumulate. Much of the data can be understood in terms of known processes once the ψ (and perhaps a small amount of charm) is included, but not all.

The large effect is small in any absolute sense, being of the order of one part in ten thousand of the production of the strongly interacting particles whose decays constitute the main experimental background to be subtracted.

The most illuminating results concern the production of pairs of oppositely charged muons or electrons. Several groups find that a substantial fraction of the leptons does come in pairs (see for example Kleberg *et al. Phys. Rev. Lett.* **37**, 1451; 1976) and that the muons are unpolarised as would be the case for electromagnetic production (Leipuner *et al. Phys. Rev. Lett.* **36**, 1011; 1976), although a Russian group has reported a large negative polarisation which is difficult to understand.

The Chicago-Princeton group (K. J. Anderson *et al. Phys. Rev. Lett.* **37**, 799 and 803; 1976; Branson *et al.* to be published) have measured muon pairs

and single muons in the same apparatus so that systematic effects in any comparison are much reduced. Readers who watched *The Key to the Universe* may remember seeing this spectrometer in action in an experiment by H. L. Anderson *et al.* (*Phys. Rev. Lett.* **36**, 1422; 1976) who built it around the 184-inch diameter magnet taken from the old Chicago cyclotron. Branson *et al.* find that 0.7 ± 0.2 of all the muons they observe come from pairs. This is near enough all of them that it will not be easy to decide whether any true single muon production takes place.

The mass spectrum of pairs from this and other experiments shows peaks for the known vector mesons (ρ , ω , φ , ψ and ψ') superimposed on a continuum which drops steeply as mass increases. Earlier suggestions of a new particle with a mass of 6 GeV have not been confirmed (Hom *et al.* *Phys. Rev. Lett.* **36**, 1236; *ibid.* **37**, 1374; 1976). It is striking how many of the pairs have masses less than the ρ or ω mesons. These are most likely due to three-body decay modes of the eta. The effect of three body decay modes of π^0 and η can be seen even more strikingly in the results of Guy *et al.* (to be published) who studied electron production in a track sensitive hydrogen target within a neon-hydrogen bubble chamber at low (4 GeV) collision energy. They find no evidence of anomalous production. (A search at 0.8 GeV/c showed no anomaly down to the level of parts in a million (Browman *et al.* *Phys. Rev. Lett.* **37**, 246; 1976)).

All low energy or low transverse momentum experiments using electrons must deal with the low mass background and it takes little imagination to realise how easy it would be to underestimate the subtraction. Nevertheless we must wait for a more direct confrontation before dismissing the signals at 10 to 24 GeV of Beier *et al.* (*Phys. Rev. Lett.* **37**, 117; 1976) and at high energy and low transverse momentum of Baum *et al.* (*Phys. Lett.* **60B**, 485; 1976), or accepting the need for Lederman and White's provocative mystery particle (*Phys. Rev. Lett.* **35**, 1543; 1975).

The smooth continuum (minus decay background) is of great interest in its own right. It is thought to be due to the annihilation of a quark in the projectile with an antiquark in the target (or *vice versa*) to give a highly virtual photon which promptly materialises as a lepton pair (Drell & Yan *Phys. Rev. Lett.* **25**, 316; 1970). The theory is very far from being clear cut but is in quite surprising agreement with the overall magnitude of the effect. As this is one of the few places where there is a direct link between electron-positron annihilation, deep inelastic scattering and the collisions of strongly interacting

particles, it will obviously repay further study. □

High pressure methods

from John Walker

THE diamond anvil high pressure cell and its calibration by a ruby crystal are currently revolutionising the study of materials under high pressure. For the first time they have enabled pressures similar to those at the centre of the Earth to be generated and measured simply and conveniently in the laboratory, thus opening up a new realm of study.

Pressure can cause dramatic changes in physical properties such as structure, electrical conductivity and transparency. The difficulty has been that high pressures could be generated only by massive (and relatively expensive) apparatus, so experiments had to be constructed around the high pressure cell. This effectively ruled out many experiments such as those involving superconductivity, where the material has to be cooled to very low temperatures. The diamond anvil cell on the other hand is compact (15 cm long) and portable and can easily be incorporated into other apparatus. Another advantage over its ponderous predecessors is its transparency to electromagnetic radiation, which makes optical and X-ray experiments much easier.

The cell was invented at the United States National Bureau of Standards by Weir *et al.* (*J. Res. natn. Bur. Stand.* **A63**, 55; 1959). It basically consists of a pair of brilliant-cut diamonds pressed together, with the material under study in between (Fig. 1). A relatively small force applied to the large faces of the diamonds results in a large pressure between the small faces. The gasket helps to retain the specimen under study, reduces the risk of shattering the diamonds, and keeps the pressure equal and isotropic throughout the sample volume.

The diamond anvil cell thus made pressure generation much more convenient. But for some time there remained the problem of measuring the pressure exerted. Previously, one way was to measure the sudden change in resistivity of materials due to pressure-induced transitions into the metallic phase, but only certain fixed points on the scale are available. Another way is to measure the change in lattice parameter of sodium chloride crystals. This technique, however, is very slow—up to two weeks to measure a single pressure—and can only be used to measure

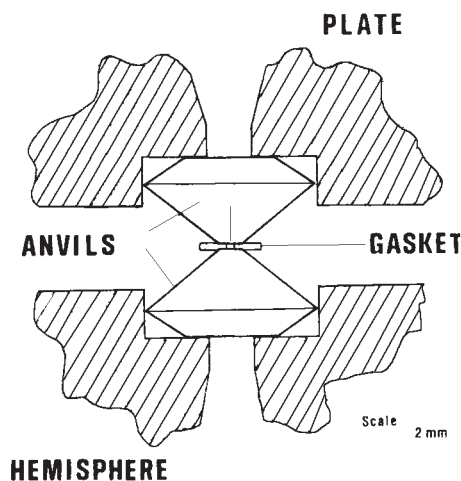


Fig. 1

pressures less than 291 kbar because of a phase change in NaCl at this pressure (1 bar = 1 atmosphere).

In 1972 however, NBS solved the problem of pressure measurement (Forman *et al.*, *Science*, **176**, 284; 1972). The system can be calibrated by measuring the shift in the R_1 ruby fluorescence line (the ruby laser line) which is linear with pressure up to at least 300 kbars (Piermarini and Block, *Rev. sci. Instr.*, **46**, 973; 1975). The fluorescence is strong and easily excited, and the shift rate is quite large ($0.036 \text{ nm kbar}^{-1}$). This technique has also led to a downward revision of the previously accepted pressure scale.

What are the fields of study that have benefited from these advances? In one study at NBS (Block and Piermarini, *Physics Today*, **29**, 44; 1976) the semiconductor-to-metal transition in silicon and gallium phosphide was observed by noting the pressure at which transparent crystals within the cell became opaque. A television camera enabled visual observations to be extended into the infrared. Simultaneous measurements of electrical resistance confirmed an order-of-magnitude conductivity change when opacity occurred.

The small size of the diamond anvil cell has great advantages in low temperature physics. One incorporated into a dilution refrigerator can apply pressures of 100 kbars at temperatures as low as 0.03 K (Webb *et al.*, *Rev. sci. Instr.*, **47**, 59; 1976), and has been used to study the way the superconducting transition temperature changes with pressure.

At the other extreme of temperature the transition of graphite to diamond has been studied at 260 kbars and 3,000 K (Ming and Bassett, *Rev. sci. Instr.*, **45**, 1115; 1974). Applications have also been reported in radioactivity, crystallography, optical spectroscopy and in the breakdown of lubricating fluids under high pressures

(as found in bearing contacts).

The diamond anvil cell can easily equal pressures inside the Earth's crust (tens of kilobars). Hence geologists find the cell useful in experimental studies. Sung (*Rev. sci. Instr.*, **47**, 1343; 1976) has used the apparatus to study the kinetics of the olivine to spinel transition. Even pressures at the centre of the earth (3.6 Mbar) may soon be routinely available. Mao and Bell (*Science*, **191**, 851; 1976) have already broken the 1 Mbar barrier.

The technology of pressure generation and measurement has up to now lagged behind that of temperature, its complementary variable; the diamond anvil cell should help it to catch up. Every laboratory should have one. □

The sequence is not enough

from N. J. Proudfoot

An EMBO Workshop on Genes and Spacers was held at Davos, Switzerland on 10–17 January, 1977. It was organised by M. L. Birnstiel and S. G. Clarkson (Institut für Molekularbiologie II, Zürich, Switzerland).

THE aim of this meeting was clearly outlined by one of its organisers, M. Birnstiel (Institut für Molekularbiologie II, Zürich). In simple terms his message was that we had perhaps heard enough about eukaryotic gene isolation and that it was high time we actually compared data from structural or functional studies made on purified genes.

Two important contributions followed that described new DNA sequencing techniques which greatly speed up that once arduous process. First, W. Gilbert (Harvard University) briefly described the now well known, although as yet unpublished, Maxam and Gilbert chemical cleavage DNA sequencing method. By labelling the end of a DNA fragment (usually obtained by restriction enzyme digestion of DNA) with ^{32}P and then breaking it at base-specific sites using various chemical tricks, the partially degraded DNA fragments could be ordered on a gel and the DNA sequence established. Second, N. Brown from F. Sanger's laboratory (MRC Laboratory of Molecular Biology, Cambridge, UK) described the plus and minus gel sequencing method originally published by Sanger and Coulson (*J. molec. Biol.* **94**, 441; 1975). This method starts by actually synthesising ^{32}P -DNA in a test tube using *E. coli* DNA polymerase.

Then with various combinations of dCTP, dTTP, dGTP and dATP (the four substrates used by DNA polymerase) the ^{32}P -DNA can be made to terminate at base-specific sites. Again, a DNA sequence may be deduced by fractionating the different DNAs on a gel. These two DNA sequencing methods have essentially increased the speed of DNA sequence analysis from the old rate of about 100 nucleotides a year to 100 nucleotides in a few 'good' days. Brown went on to show the essentially complete sequence of the bacteriophage ΦX174 , 5,375 nucleotides in length at the last count. Happily for Sanger's group, their massive sequencing effort has been rewarded by the discovery of two overlapping gene systems, in which two proteins are read off the same DNA sequence, but in two different phases. That genes *E* and *D* of ΦX overlap has recently been published by Barrell, Air and Hutchison (*Nature* **264**, 34; 1976). Brown reported that two further genes *A* and *B* also overlap, so that two examples of this bizarre phenomenon occur in the same bacteriophage. Of course it remains an open and exciting question as to whether or not overlapping genes are present in other prokaryotic or eukaryotic organisms.

Two of the first eukaryotic genes to be isolated, frog 5S and sea urchin histone genes, both repeated many times over in tandem, are now well on the way to being completely sequenced. D. Brown (Carnegie Institution, Baltimore) gave an elegant report on the former system. After describing electron microscopy and restriction enzyme data on this DNA, he presented the complete sequence of the spacer in 5S DNA, showing very nicely the satellite 15-nucleotide repeat sequence originally discovered by Brownlee, Cantwright and Brown (*J. molec. Biol.* **89**, 703; 1974) and demonstrating how variation in the number of these repeats accounted for the variation in the absolute length of these genes. He went on to describe the sequence of the apparent promoter for 5S RNA, just beyond the spacer region, but apart from a repeated sequence of 10 nucleotides, no clues to its identity were observable. Several other possible eukaryotic promoter sequences, in yeast *rDNA* and SV40 DNA, were described by W. Gilbert and W. Fiers (Gent). Already it is becoming painfully obvious that no promoter-specific homologous sequences are generally present in eukaryotes. Histone genes from two different species of sea urchin were described by M. Birnstiel and L. Kedes (Stanford University). Long stretches of sequence from both coding sequences and spacers were presented, but even after computation (described by H. Smith (Johns Hopkins Univer-

sity, Baltimore)) little sense could be made. One of the great hopes for the histone gene system is that control signals before and after each of the six coding or mRNA sequences (present in each histone gene repeat unit) may be recognisably similar and that these control signals may be conserved between different species. But the data is as yet too fragmentary to be sure whether or not such hopes are justified. One such control signal, however, does appear to be generally present in eukaryotes. Proudfoot and Brownlee (*Nature* **263**, 211; 1976) recently described a sequence A–A–U–A–A–A presented in six different eukaryotic mRNAs close to their 3'-terminal poly(A) sequences. Fiers described the same sequence close to the poly(A) of EMC viral RNA and M. Billeter (Institut für Molekularbiologie, Zürich) suggested that this sequence was near the poly(A) of the related RNA tumour viruses RSV and AMV.

J. Rubin (Stanford University) presented some intriguing results on a gene from *Drosophila* of unfortunately unknown function. He described how the DNA sequences on either side of the transcribed positions of this gene appear to be nearly identical for as many as 500 nucleotides, on the basis of hybridisation data using restriction fragments and an ingenious heteroduplex experiment. More understandable were the interesting results reported by Gilbert on RSV. He described the sequence of complementary DNA made using the RSV RNA template with its natural tRNA primer and reverse transcriptase. This DNA, about 100 nucleotides long, is complementary to the 5'-end of the viral 30S RNA genome. For a long time the question has been, how is the rest of the virus copied into DNA, an essential requirement for viral integration into the host's genome. Gilbert's answer is simple. The sequence at the 3'-end of the virus contains a region of 19 nucleotides identical to the 5'-end sequence. Thus the tRNA primer may generate a second DNA primer allowing reverse transcriptase to copy the whole viral RNA.

With a few notable exceptions, the main lesson learnt from the extensive sequence data described at Davos is that knowing the sequence is not enough. Major breakthroughs must be made before any understanding of the relationship of sequence to function is achieved.

I will finish this selective account by describing two such possible breakthroughs. First, Gilbert described the use of various chemical reagents that modify DNA to locate the exact binding position of a particular protein on DNA. His model system was as usual the *lac* operon, *lac* repressor complex.

He showed very clearly, using a rather fearsome phosphate-specific reagent, that the *lac* repressor only binds on one side of the double helix. Second, both E. De Robertis (MRC, Cambridge) and D. Brown described experiments done in J. Gurdon's laboratory at Cambridge, which involved the injection of either frog 5S DNA or *Drosophila* histone DNA into the nuclei of frog oocytes (a seemingly impossible manipulation). Both experiments suggested that the foreign DNAs were transcribed specifically to generate in the first case normal 5S RNA and in the second histone proteins, detected by two-dimensional fractionation techniques. These exciting results suggest that frog oocytes may well provide an invaluable assay system for purified eukaryotic gene expression. □

Nova Sagittae 1977 and Vulpeculae 1976

from David W. Hughes

TENACITY and a truly astronomical memory are two attributes specifically required by the amateur astronomers who successfully search the sky for novae. Tenacity, because discoveries are few and far between—about 300 hours of painstaking observation being required to discover a nova. The encyclopaedic memory is also essential, the relative positions and brightness of about 10,000 stars must be known so that the interloper, the star which has suddenly and dramatically increased in brightness, can be easily recognised.

The last two novae found have been discovered by British amateur astronomers, the most recent one by John Hosty, a Huddersfield postman, on 7 January, 1977 in the constellation of Sagitta (the actual position is right ascension $19^{\text{h}}37^{\text{m}}08.18^{\text{s}}$, declination $+18^{\circ}00'58.2''$, equinox 1950.0). This has been reported in *Circulars* 3025/7 and 3030 of the *International Astronomical Union's Central Bureau for Astronomical Telegrams*. It was discovered at a magnitude of 7.2 (about three times less bright than stars visible to the naked eye, which have magnitudes less than 6.0) and has been getting steadily fainter since its discovery, as shown in Fig. 1. Most novae will decrease in brightness by about 3 magnitudes from their maximum value in about 40 d and then continue getting fainter at a slightly diminished rate.

The previous nova discovery was by George Alcock, a retired school master from Peterborough, his nova being in Vulpecula (right ascension $19^{\text{h}}27^{\text{m}}.04^{\text{s}}$, declination $+20^{\circ}21'43''$) and was near its maximum brightness at magnitude 6.5 at discovery (*IAU Circular No.* 2997). As seen in Fig. 1 the nova

became 3 magnitudes fainter than maximum in 42 d and 6 magnitudes fainter in about 68 d. Interestingly both novae are in the Milky Way and are very close to each other in the sky.

The instruments used to detect novae are relatively simple in comparison to other astronomical apparatus. Both Hosty and Alcock used binoculars, in fact Hosty used only half a pair of binoculars as the other half had been unfortunately broken at some time. The two instruments were 10×50 and 15×80 respectively, the first figure being the magnification and the second figure the objective lens diameter in millimetres. They would have fields of view of about 6° and 4° in diameter, the Hosty instrument detecting stars down to about 10th magnitude under excellent sky and seeing conditions. The field of view would contain, on average, 30 stars of 8th magnitude and just over 200 brighter than 10th. The daunting task of the nova hunter is to pick out the new star as he sweeps slowly over the 10,000 or so stars brighter than 8th magnitude that can be seen from a good observing sight at any one time.

What is the use of discovering novae? Well, apart from the sudden rise to fame for the finder, these stars are of considerable intrinsic interest, being at a fascinating stage in their evolutionary history. Novae occur just before a star finally dwindles to become a white dwarf. All the nuclear energy-producing reactions converting hydrogen and helium into higher atomic number elements have ended, and the star is getting its energy simply from the gravitational potential energy that is slowly released as it shrinks. The pre-nova star is typically of spectral type O or B, corresponding to a surface temperature of 20,000 to 40,000 K, with a radius about 0.3 solar radii and a mass between 0.2 and 2.5 solar masses. The absolute magnitude is around +4.5. On explosion the magnitude jumps by 11, equivalent to an increase in brightness of about 25,000 times. During the outburst the star loses on average 0.001 solar masses, the energy emitted in the few days of outburst being about 10^{45} ergs, corresponding to the energy emitted by the Sun over 2,000 yr. From spectral observations it can be seen that the luminous layers of the star are expanding outwards at velocities around 1,000 to 2,500 km s^{-1} in a rather non-uniform fashion, causing the superposition of absorption and emission features in the line spectra. At maximum the nova reaches an absolute magnitude of about -8.0 the expanding gas cloud having a radii of about 100 solar radii. After maximum the nova slowly fades into insignificance, back to the same magnitude as the pre-nova star. By care-

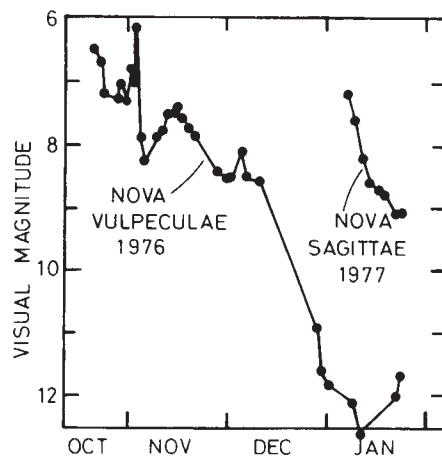


Fig. 1 The visual magnitude of the novae as a function of time. A change in magnitude by one unit is produced by a change in brightness by a factor of 2.51.

fully searching the Palomar Sky Survey photographic plates (*IAU Circular* 2998) the pre-nova star of nova Vulpeculae has been found and is a blue star of photographic magnitude 18.3. A search for the pre-nova of Sagittae 1977 has revealed three stars at the plate limit of about 20th photographic magnitude within $\sim 1''$ of the nova's position.

In the Galaxy there may be about 50 novae a year, only a few (2.2 yr^{-1} on average) being discovered, the rest being hidden behind dense interstellar dust clouds or being too faint at maximum.

The general excitement caused when one is discovered and the wealth of knowledge gained about this fascinating stage of stellar evolution is ample justification for the arduous and tireless search that worthy amateur astronomers like Hosty and Alcock conduct. It is very unfortunate however that so little is known about the pre-nova state of the star. So far only one spectrum, that of V603 Aquilae, has been recorded before the star became a nova. Our knowledge of what causes a star to suddenly explode in this unexpected way is thus pitifully small.



A hundred years ago

PROF. DIETERICI, of Berlin, sought to show in a public lecture, delivered a few days since, that the theories of Darwin were by no means novel, having been essentially published by learned Arabs in the tenth century. From *Nature* 15, February 8, 326; 1877.

review article

Octopamine

Julius Axelrod & Juan M. Saavedra*

The sympathomimetic amine octopamine (p-hydroxyphenylethanolamine) has the properties of a neurotransmitter in many invertebrate species. In mammals it seems to be a cotransmitter with noradrenaline.

THE actions of neurotransmitters are ubiquitous, influencing a variety of tissues and physiological functions. For a compound to be considered a neurotransmitter, it should be present in nerves, together with its biosynthetic enzymes. When the nerves are stimulated, the transmitter should be discharged from nerve endings. Once liberated, the transmitter should be recognised by and bind to its specific receptor on the postjunctional cell membrane and produce a biological response characteristic of that cell. Mechanisms should be available to terminate rapidly the actions of the neurotransmitter. Until recently, only acetylcholine and noradrenaline were established as neurotransmitters. However, many compounds, such as dopamine, 5-hydroxytryptamine (5-HT), histamine, gamma aminobutyric acid, certain amino acids and small peptides meet many of the criteria necessary for chemical neurotransmission. Recent work has indicated that octopamine fulfils the requirements for a neurotransmitter, especially in invertebrates.

Octopamine (*p*-hydroxyphenylethanolamine) was first detected in the venom-producing posterior salivary gland of *Octopus vulgaris* by Erspamer and Boretti¹. Soon afterwards, Armstrong and co-workers noted that relatively large amounts of the deaminated metabolite of octopamine, *p*-hydroxymandelic acid, was present in the urine of man and other mammals². However, with the methods available at that time (paper chromatography) octopamine could not be detected in tissues unless a monoamine oxidase inhibitor was previously administered³. The injection of radioactive tyramine into rats led to the formation of radioactive octopamine which was highly localised in nerves containing noradrenaline⁴. Stimulation of the splenic nerves of cats previously given ³H-tyramine resulted in the release of ³H-octopamine⁵. It was also observed that dopamine- β -hydroxylase, the enzyme that converts dopamine to noradrenaline⁶, and highly localised in sympathetic nerves⁷ also hydroxylates tyramine to octopamine *in vitro*⁸. These observations suggested that octopamine, the monohydroxylated analogue of noradrenaline, might be a neurotransmitter.

The development of a sensitive enzyme isotope method for measuring octopamine in tissues made it possible to study its normal occurrence, physiological disposition and metabolism⁹. The method depends on the ability of phenylethanolamine *N*-methyltransferase to transfer the methyl group of ¹⁴C or ³H-methyl-*S*-adenosyl-L-methionine to octopamine present in tissues. Phenylethanolamine *N*-methyltransferase is highly localised in the adrenal medulla and can *N*-methylate noradrenaline as well as other β -hydroxylated phenylethanolamines, including octopamine¹⁰. A partially purified enzyme is incubated together

with aqueous tissue extracts and radioactive *S*-adenosyl-methionine. The radioactive *N*-methyl octopamine (synephrine) formed is then separated from other interfering compounds by selective extraction into a non-polar solvent (mixture of toluene and isoamyl alcohol) and the radioactivity measured directly in the solvent⁹ or coupled with paper chromatography of the *N*-methylated amines¹¹. These methods have been found to be specific, rapid and very sensitive. As little as 20 pg can be measured using ³H-methyl-*S*-adenosyl-L-methionine of high specific activity¹².

Sensitive assays using specific methyltransferase enzymes and extraction by appropriate solvents and ³H-methyl-*S*-adenosyl-L-methionine have been used to measure a number of biogenic amines in the picogram range. 5-HT is measured by acetylation with liver *N*-acetyltransferase followed by *O*-methylation with pineal hydroxyindole-*O*-methyltransferase¹³, an enzyme that specifically *O*-methylates *N*-acetyl-5-HT. Noradrenaline and dopamine are *O*-methylated by catechol-*O*-methyltransferase and the ¹⁴C methylated amine separated by cleavage of the β -hydroxylated amine with periodate¹⁴. Histamine is assayed by a highly specific histamine *N*-methyltransferase from the brain¹⁵. An enzyme from the lung is used to methylate tryptamine¹⁶. Phenylethylamine¹⁷ and tyramine¹⁸ can be measured by hydroxylation with dopamine- β -hydroxylase followed by methylation with phenylethanolamine *N*-methyltransferase.

Octopamine in mammals

Using the isotope enzyme assay, octopamine has been found in the peripheral tissues and brain of rats and other mammals (Table 1)^{19,20}. It is present in sympathetically innervated organs such as the heart, spleen and salivary gland. With a radiochemical enzymatic assay which allows the simultaneous determination of *p*-octopamine and *m*-octopamine, equal amounts of these amines were found in rat salivary glands (H. A. Robertson, A. A. Boulton, J. C. David and T. J. Danielson, personal communication). In the cat's heart, octopamine is present mainly in the atria. Octopamine is also found in the brain of all mammalian species examined, including man¹². It is unequally distributed in the brain; highest values are present in the hypothalamus, midbrain and spinal cord. The pineal gland had the greatest levels of octopamine.

In the foetal rat brain octopamine can be detected after 15 d of gestation²¹. Up to 17 d of gestation, octopamine is the predominant amine in the foetal rat brain and is twice as high as that of noradrenaline. After 18 d of gestation, there is a precipitous decline of octopamine levels in the foetal rat brain, and this coincides with a rise in monoamine oxidase. The administration of phenylalanine together with drugs that block the hydroxylation of this amino acid results in

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Table 1 Distribution of octopamine in tissues

	ng g ⁻¹	References
Rat		
Heart	47	20
Salivary gland	96	19
Spleen	23	19
Pineal	450	20
Brain (adult)	3-12	11, 20, 24
Brain (foetal)	26	21
Human brain		
Hypothalamus	80	12
Temporal lobe	19	12
Caudate nucleus	11	12
Raphe nucleus	8	12
Frontal lobe	5	12
Parietal lobe	2	12
Cat heart		
Atria	100	20
Ventricle	10	20
Rabbit heart	135	20
Brain	7.5	11
Mouse heart	14	30
Brain	14	11
Goldfish heart	7	20
Octopus		
Circumoesophageal ganglia	1200	34
Superior basal lobe	4600	34
Stellatum ganglia	21	34
Posterior salivary gland	1,310,000	34
Frog heart	0	20
Lobster		
Brain	226	37
First thoracic nerve cord	830	37
Abdominal nerve cord	45	37
Peripheral nerve bundle	11	37
Earthworm		
Cerebral ganglia	7390	42
Ventral nerve	5340	42
Gut	1060	42
Muscle	233	42
Firefly lantern	125	43
Cockroach		
Abdominal cord	1010	40
Thoracic nerve cord	940	40
Brain	3950	40
<i>Aplysia californica</i> ganglia		
Abdominal	35	36
Cerebral	280	36
Pedal	160	36
Pleural	30	36

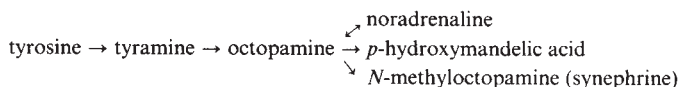
increases in the ratio of octopamine to noradrenaline from 2 : 1 to 12 : 1 in foetal rat brain.

Evidence that octopamine is present in nerves of peripheral tissue^{19,20} and brain²² was obtained by surgical and chemical denervation of sympathetic nerves. Denervation of the noradrenergic nerves of the rat salivary gland results in a partial disappearance of octopamine in this tissue. After the administration of 6-hydroxydopamine, a compound that destroys catecholamine-containing nerves, there is a marked reduction of octopamine in the rat heart, spleen, salivary gland and pineal gland^{19,20}. An intracisternal injection of 6-hydroxydopamine causes a marked depletion of octopamine in all areas of the rat brain²². In the rat heart, octopamine is found in the same fraction of a sucrose density gradient as noradrenaline-containing vesicles²⁰. All of these observations indicate that octopamine is highly localised in noradrenaline-containing neurons. Octopamine is present in small amounts relative to noradrenaline, yet it has a high rate of turnover in tissues, about six times that of the catecholamine²⁰. Thus, the higher steady-state concentration of noradrenaline as compared to octopamine may not reflect the functional importance of each amine. Though the tissue concentration of octopamine is only a fraction of that of noradrenaline and 5-HT, the amount of the major metabolites of these amines, *p*-hydroxymandelic acid (from octopamine), 3-methoxy-4-hydroxymandelic acid (from noradrenaline and adrenaline) and 5-hydroxyindoleacetic

acid (from 5-HT) are extracted in the urine of humans to the same extent^{2,23}. This suggests rapid turnover of octopamine in man.

The biosynthesis and metabolism of endogenous octopamine in the rat and mouse brain has been elucidated by the administration of precursors and enzyme inhibitors^{9,11,24,25}. An injection of a monoamine oxidase inhibitor causes more than fivefold elevation of octopamine in rat and mouse brain and peripheral tissues, indicating that deamination by monoamine oxidase is an important pathway for its metabolism^{11,24}. A minor route of transformation *in vivo* is hydroxylation to noradrenaline²⁶. The enzyme carrying out the latter reaction is localised in the liver microsomes²⁶. Another minor pathway of metabolism of octopamine is *N*-methylation to synephrine by phenylethanolamine *N*-methyltransferase¹⁰. Tyrosine or tyramine together with a monoamine oxidase inhibitor result in a much greater increase in octopamine in rat²¹ and mouse¹¹ brain than do monoamine oxidase inhibitors alone. In rats treated with a dopamine- β -hydroxylase inhibitor there is a considerable fall in the endogenous levels of octopamine in rat, brain, heart, and spleen²⁴. An unusual metabolic route for the formation of octopamine is dehydroxylation of catecholamines²⁴. The administration of L-dopa to rats leads to the increase of octopamine in the rat brain and urine of man. Dehydroxylation of catecholamines to octopamine was also demonstrated in germ-free rats, indicating that this transformation is not due to metabolism in the intestinal flora. Further evidence for dehydroxylation of catecholamines was obtained by the formation of octopamine and synephrine after an intraventricular injection of either ¹⁴C-dopamine or ³H-noradrenaline into the rat brain²⁷.

From the experiments described above, the pathway for the formation and metabolism of octopamine proceeds as follows



The release of octopamine from nerves was initially demonstrated by infusion of the radioactive amine into the cat spleen³. On electrical stimulation of splenic nerves there was a discharge of ³H-octopamine into the perfusing fluid. Infusion of ³H-tyramine into the spleen also resulted in the release of ³H-octopamine. These findings indicate that tyramine is taken up by neurons where it is β -hydroxylated to octopamine in the nerves. When these nerves are stimulated, the ³H-octopamine is released presumably by exocytosis in the same manner as noradrenaline. Release of endogenous octopamine by nerves has been also demonstrated²². After stimulation of the splenic nerve of the cat there is a discharge of endogenous octopamine and noradrenaline²². The amount of octopamine released is only about 2-5% of that of noradrenaline. It is unlikely that octopamine and noradrenaline exist in separate neurones in the spleen and it is probable that octopamine is released from nerves as a cotransmitter together with noradrenaline. Octopamine has also been reported to coexist with noradrenaline and serotonin in vesicles of pineal adrenergic nerves²⁸. After the administration of a monoamine oxidase inhibitor the amount of octopamine discharged from the cat spleen is 30-40% of the amount of noradrenaline²². The relatively large release of octopamine, a compound with weak pressor activity provides a mechanism for the blood pressure lowering actions of monoamine oxidase inhibitors.

Like noradrenaline, octopamine can be taken up by nerve endings of the rat brain by a temperature- and sodium-dependent process, and this process is inhibited by drugs

that prevent the uptake of catecholamines^{29,30}. When the brain tissue is stimulated electrically octopamine previously taken up is released. The uptake of octopamine by nerve terminals might serve as a means for rapidly terminating the actions of this amine.

In mammals, octopamine causes a transient elevation of blood pressure. The physiological actions of octopamine are similar to those of noradrenaline but it is only about 1/50 as effective³¹. Monoamine oxidase inhibitors elevate octopamine in nerves six times as rapidly as noradrenaline²⁰ and cause a disproportionate release of octopamine²² and in a reduced response. Thus, octopamine because of its weaker physiological action, may modify the actions of noradrenaline.

The administration of phenylalanine and phenylalanine hydroxylase inhibitors, which results in high increases in tissue levels of octopamine^{11,24}, produces symptoms analogous to those of phenylketonuria. Thus, the symptoms of this disease might result from an accumulation of octopamine in the brain at the expense of noradrenaline. There is an elevation of octopamine in hepatic failure which may be associated with some of its neurological and cardiovascular pathology³². The levels of octopamine in the hypothalamus of children who died of acute hepatic encephalopathy (Reye's syndrome) are 12 times higher than that of age-matched controls³³. It has been postulated that octopamine might accumulate as a false neurotransmitter and cause some of the pathology in these hepatic diseases³².

Octopamine in invertebrates

Highest concentrations of octopamine have been found in the nervous system of octopus and other cephalopods (Table 1)³⁴. The posterior salivary gland of the octopus contains as much as 1.3 mg g⁻¹ octopamine. Subcellular studies showed that octopamine is localised in synaptosomes (nerve ending fractions) of the nerves³⁴.

Octopamine is found in relatively high amounts in single identified neurones of the *Aplysia californica* (Tables 1 and 2)^{35,36}. In some nerve cells of the *Aplysia*, octopamine is present together with other putative neurotransmitters such as acetylcholine, serotonin and histamine but not dopamine or noradrenaline (Table 2). These findings indicate that in *Aplysia*, Dale's principle (one neurotransmitter for one nerve) may not hold. From these observations it is apparent that neurones containing octopamine can also exist in the absence of catecholaminergic neurones.

The nervous system of the lobster is rich in octopamine (Table 1)^{19,37,38}. The amine is highly localised in the thoracic nerve cord²⁰ and is associated with groups of neurones along the proximal portions of the second root leaving the thoracic ganglia³⁸. These octopamine-containing cells have the morphological appearance of neurosecretory cells³⁹ and receive synaptic inputs from the cholinergic nerves.

Relatively large amounts of octopamine are present in the central nervous system of the cockroach⁴⁰, locust⁴¹, earthworm⁴² and firefly lantern (Table 1)⁴³ and probably in nerves of many other invertebrates not yet examined.

Injection of ¹⁴C-tyrosine into lobster nerve tissue results in the formation of radioactive octopamine and dopamine but not noradrenaline³⁷⁻³⁹. Dopamine- β -hydroxylase (the enzyme that converts dopamine to noradrenaline, and tyramine to octopamine), tyrosine hydroxylase and aromatic amino acid decarboxylase are also present in lobster nerves³⁸. The ability of lobster nerves to synthesise dopamine and octopamine can best be explained by the occurrence of two types of nerve cells: octopaminergic cells that contain aromatic amino acid decarboxylase and dopamine- β -hydroxylase but not tyrosine hydroxylase and

dopaminergic cells containing tyrosine hydroxylase and dopa decarboxylase but not dopamine- β -hydroxylase³⁸. Thus nerves containing octopamine and nerves containing catecholamine seem to be spatially separated.

Octopamine-containing root cells of the lobster can synthesise ³H-octopamine from ³H-tyrosine³⁹. When these neurones are depolarised with high concentrations of potassium there is a marked release of ³H-octopamine³⁹. The discharge of octopamine by potassium is highly dependent on Ca²⁺. Thus, the octopamine-containing cells of the lobster exhibit the properties associated with the release of a neurotransmitter from nerves; liberation by depolarisation and a requirement for Ca²⁺.

Table 2 Coexistence of octopamine and other putative neurotransmitters in single identified neurones of *Aplysia californica*

	R-2	R-14	L-11	C-1
Octopamine	2.5×10^{-6}	1.5×10^{-4}	9.1×10^{-6}	ND
5-HT	1.8×10^{-5}	3.4×10^{-5}	1.1×10^{-5}	9.4×10^{-4}
Dopamine	ND	ND	ND	ND
Noradrenaline	ND	ND	ND	ND
Acetylcholine	3.9×10^{-4}	ND	3.3×10^{-4}	ND
Histamine	3.0×10^{-6}	7.0×10^{-6}	4.5×10^{-6}	1.4×10^{-5}

Results are expressed in molarity. Molarity was calculated from volume estimation obtained by measurements of greatest and smallest cell diameters at the time of dissection (from Brownstein *et al.*³⁶). ND, None detected.

Octopamine has been shown to have potent effects in insects. The local application of octopamine to the nerve cord of the cockroach causes an increase in cyclic AMP levels⁴⁴. This, in turn, produces a stimulation of phosphorylase and a pronounced glycogenolytic effect⁴⁵. Adenylate cyclase in cells of thoracic ganglia of the cockroach, that can be specifically stimulated by low concentrations of octopamine, has been identified⁴⁴. Octopamine, dopamine and 5-HT are additive in stimulating adenylate cyclase activity suggesting that each of these amines activates separate receptor sites. Octopamine was found to be the most potent endogenous amine in eliciting the light-flash in the firefly lantern⁴⁶. In locust and grasshoppers, octopamine inhibits the rhythmic contractions of muscles just as stimulation of neurones synthesising octopamine does^{41,47}.

The cyclic AMP levels in several lobster ganglia are elevated by octopamine which produces a characteristic pattern of nerve firing in certain ganglia cells⁴⁸. When octopamine is perfused across the opener muscle in the walking leg of the lobster it causes a prolonged contraction³⁹. This effect is stereospecific: the D(-) isomer of octopamine is 50 times more potent than the L(+) isomer.

Ionotropic application of octopamine to the nervous system of *Aplysia* revealed the presence of receptors that are very sensitive to octopamine and mediate a hyperpolarising-conductance increase in response⁴⁹. These receptors showed a marked preference for octopamine and had an absolute requirement for a β -hydroxyl group. Cells in the cerebral ganglia are most responsive. These cells whose processes are localised in the neurophile where functional synapses are present have high concentrations of octopamine^{35,38}.

Incubation of the abdominal ganglia of *Aplysia* with octopamine caused a considerable elevation in cyclic AMP⁵⁰. This increase is prevented by the adrenergic blocking agent phentolamine, and by methylsergide. Incubation of the ganglia with octopamine also resulted in an incorporation of P³² into a specific protein⁵¹. The change in phosphoprotein metabolism produced by octopamine and mediated by cyclic AMP seemed to be localised to the neurophile.

Conclusion

Octopamine is highly concentrated in neurones of several invertebrate species. Unlike in mammals, octopaminergic neurones in invertebrates are spatially separated from catecholaminergic neurones. In identified nerve cells of *Aplysia*, however, this amine coexists with other putative neurotransmitters. Octopamine is synthesised in nerves from tyrosine and tyramine and metabolised mainly by monoamine oxidase. When lobster nerves are depolarised, octopamine is liberated by a Ca^{2+} -dependent process. A specific adenylate cyclase is stimulated by octopamine in several invertebrates to activate phosphorylase in the cockroach, induce a light-flash in firefly lantern or inhibit rhythm contractions in locust muscle. All of these observations provide compelling evidence that octopamine is a neurotransmitter in invertebrates.

In mammals octopamine is localised in nerves in peripheral tissues and brain where it seems to coexist with noradrenaline, the catecholamine being present in much higher concentrations. Octopamine is released from nerves together with noradrenaline and it may under certain conditions modify the actions of the adrenergic neurotransmitter. Octopamine is present in unusually high concentrations in certain neurological and hepatic diseases and may have a pathophysiological role.

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articles

Haloes from plutonium minerals and fission alphas?

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The normal alpha decay of naturally occurring ^{244}Pu can be expected to have produced pleochroic haloes detectable in existing rocks. Several methods of identifying such haloes are described here. Giant haloes might also be produced by the α particles that occasionally accompany the spontaneous fission of ^{244}Pu . It is plausible to speculate that in ideal circumstances such giant haloes might be as large as 100 μm , and that the X rays recently induced from central inclusions of giant haloes and identified as L rays of Z — 126 might instead have been K X rays from Te fission fragments.

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BECAUSE of its relatively short half life of 82.8 Myr, ^{244}Pu is present only in small amounts in the contemporary Earth. Mass spectrometry of meteorites, however, shows that at the time the Earth formed the ratio of $^{244}\text{Pu}/^{238}\text{U}$ was about 0.014 (refs 1 and 2). Consequently, for a period in the Earth's history there was sufficient ^{244}Pu present to affect the economy of fissionable materials and to form minerals. Isotopes which are products of the fission of ^{244}Pu have been identified in meteorites and the Earth^{3–5}, and work has begun on the mineral chemistry of plutonium⁶. It would be interesting to know the enrichments, fractionations, crystallisations and mineral formations of which plutonium is capable in geochemical processes. In the past such minerals may have been concentrated sufficiently to produce normal and anomalously large pleochroic haloes. Identification and study of these haloes might contribute to more accurate estimates of the concentration and the nature

of plutonium minerals at different times in the Earth's history.

Pleochroic haloes are spherical shells of coloration usually about 40 μm thick surrounding small grains of radioactive mineral. They commonly occur in pegmatites around grains of minerals such as sphene, apatite, zircon and monazite containing uranium and thorium. Haloes should also have formed around grains that have in the past contained sufficient amounts of plutonium minerals. Although haloes occur in several different types of rock, they have been studied mostly in biotite mica largely for reasons of experimental convenience⁶⁻¹⁰.

Haloes generated by 10^{10} to 10^{11} atoms of ^{244}Pu would only rarely be distinguishable visually from haloes generated by ^{238}U or ^{232}Th . But plutonium haloes might be distinguishable by mass spectrometric detection of residual ^{241}Pu , its descendant ^{236}U , or by the presence of characteristic fission fragments in the central inclusions.

An interesting possibility is that some haloes around inclusions containing more than 10^{15} atoms of ^{244}Pu might be larger than uranium and thorium haloes. For every 3.2×10^5 decay α particles with energies around 5 MeV, an inclusion of ^{244}Pu will produce one α particle accompanying a spontaneous fission. More than half of these spontaneous fission α particles will have energy greater than 15 MeV and might produce shells of coloration with thicknesses as great as 100 μm .

The possible existence of plutonium haloes is interesting not only as further evidence of the presence and activity of a mineralogy of primordial ^{244}Pu in geological processes. The subject has interest because it may be possible to identify as ^{244}Pu haloes the 'giant haloes' reported by Gentry¹⁰. Such an identification might provide a basis for an alternative interpretation of the data from which Gentry *et al.*¹¹ deduced the existence of elements $Z = 116, 124, 126$ and 127 —the so-called superheavy elements.

Normal haloes

Halo coloration behaves like the photographic process. There is a threshold of exposure below which no colouring occurs; there is a region in which optical density of the mica increases approximately linearly with exposure; above certain exposure levels there is saturation, and coloration remains constant. Finally, very intense exposures will produce complicated bleaching and reversal. Deutsch⁷⁻⁹ studied naturally occurring uranium and thorium haloes in various biotite micas and also artificially induced coloration in these micas with α particles from radon. The coloration is triggered by sufficient ionisation density, but the mechanism is not known. They concluded that a fluence (time integrated flux) of about 10^{13} α particles per cm^2 is required to initiate coloration in the laboratory. They also found that natural fluences must be four to seven times greater than artificial ones to make the same effect in a given mica, and that different micas may differ widely in sensitivity⁷⁻⁹.

Since the fluence must exceed a threshold for coloration to occur, no halo will form beyond the range of the most energetic α particle. Also, if the fluence drops below $\sim 10^{13}$ α particles per cm^2 there may not be sufficient ionisation to colour the halo. Consequently, the size of the halo is limited by the range of the α particles or by inverse square dissipation of the fluence below the threshold, whichever happens first.

Under the assumption that the fluence threshold in nature is 4×10^{13} α particles per cm^2 , the minimum number of α particles that will colour a halo out to 18 μm from an inclusion small compared with the halo size is 1.6×10^9 . This number of α particles would be produced by the decay of 5.4×10^8 nuclei of ^{244}Pu , and if they occupy 10% of the volume of the inclusion, 1 μm is the characteristic dimension of the smallest inclusion for which the size of its halo is determined by the range of α particles rather than by the inverse square law. In Fig. 1 such haloes correspond to inclusions with initial amounts of ^{244}Pu

above the horizontal line labelled 'threshold of formation of visible haloes'.

Can such haloes be present in existing rocks? Can more than 5×10^8 atoms of plutonium have been concentrated into mineral grains 1 μm in diameter or larger? The likelihood of such an occurrence depends on the extent of geochemical concentration of plutonium at different geological times, and on the abundance of ^{244}Pu at the time of the formation of the mineral grains.

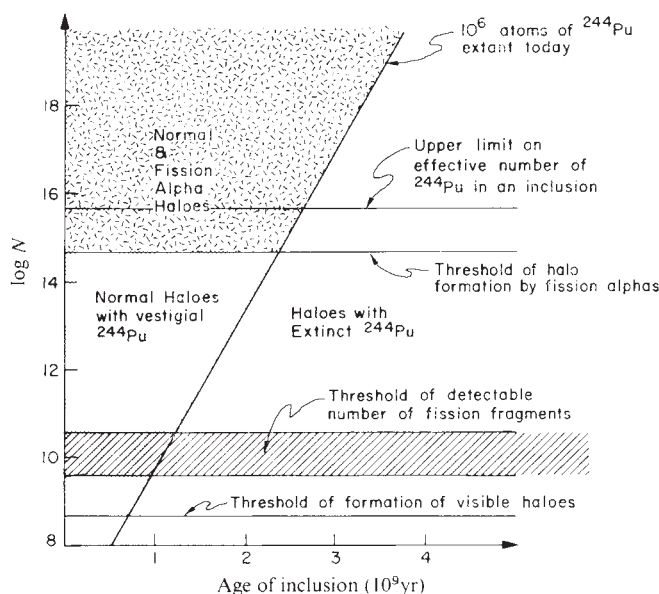


Fig. 1 Relationship of number of ^{244}Pu atoms in a mineral inclusion to the age of the inclusion. The diagonal line corresponds to 10^6 atoms of ^{244}Pu at present extant in the inclusion. The other features of the graph are discussed in the text. N is the number of ^{244}Pu atoms in inclusion at the time of formation.

Ores and minerals of most elements can be found somewhere in nature with enrichments of at least 10^6 – 10^8 times their average abundance in the Earth. Very pure grains of minerals and, in some cases, the elements themselves, can occur within such ores. The frequency of occurrence of the enriched ores and grains depends on the elemental abundance and the particular chemistry of the minerals. Hoffman *et al.*¹² examined some ore rich in bastnaesite, $(\text{Ce}, \text{La})\text{FCO}_3$, from Mountain Pass, California, in which cerium is some 10^6 times more abundant than its average in the Earth. In 8.5 kg of the bastnaesite, they found 2×10^7 atoms of ^{244}Pu which they estimate is an enrichment of 2×10^8 over the average amount present in the Earth.

Moreover, bastnaesite may not be the best mineral in which to look for the maximum concentration. Considerably greater concentrations of plutonium probably occur in monazite. One may expect plutonium to concentrate approximately as thorium because the most stable oxidation state of the two chemicals is four, and in this they differ from all other lanthanides and actinides. But thorium is only 0.39% of the Mountain Pass bastnaesite¹³ while it occurs as up to 28% of some monazites¹⁴. (Monazites from Mountain Pass are about 3% thorium.) Furthermore, monazite is a phosphate mineral, and during formation of phosphates of uranium, thorium and plutonium, the fractionation of plutonium is favoured by a factor of two to three (ref. 5).

The abundance of ^{244}Pu drops by 10^6 every 1.65×10^9 yr. Thus, pleochroic haloes will be produced by plutonium far enough back in geological history that the combination of concentrating processes and abundances suffices to produce

grains 1 μm in diameter, or larger, containing 5.4×10^8 or more atoms of ^{244}Pu . Whether this occurred in rocks young enough to be part of the Earth's crust today needs to be determined experimentally.

If, when examined in thin sections, samples exhibited haloes with sharp rings and had only one radioactive element, Th, U or Pu, in the centre, they could be distinguished easily. Ideally the three α particles emitted as a ^{244}Pu nucleus decays to ^{232}Th would produce rings of coloration in biotite at about 14.2, 14.6 and 17.8 μm from the surface of the inclusion. Among the bands of colour that uranium would produce would be two at 15.3 and 19.9 μm . Two of thorium's several colour bands would occur at 12.4 and 20 μm . Unfortunately, inclusions can be expected to contain all three elements. Furthermore, the rings are not sharp except under rare ideal circumstances and are usually seen as bands or even discs of colour. Colour bands produced by plutonium will normally be difficult to distinguish from those from uranium. If no uranium were present, it might be possible to recognise a combination plutonium–thorium halo. It might be possible with such a combination to reproduce the characteristics of the X haloes mentioned by Gentry⁶.

A more certain identification of plutonium haloes would be the mass spectrometric detection of vestigial ^{244}Pu , its descendant ^{236}U or fission fragments in a halo inclusion. Detection of plutonium with a mass spectrometer requires the presence in the inclusion of about 10^6 atoms. Their presence will depend upon the age of the inclusion and the original amount present. The diagonal line in Fig. 1 corresponds to inclusions containing 10^6 atoms of ^{244}Pu today. Therefore, points in the stippled region represent inclusions which should still have detectable amounts of ^{244}Pu . For example, an inclusion formed 1.4×10^9 yr ago must have contained 10^{11} atoms of ^{244}Pu in order to contain 10^6 atoms today. Points to the right of the diagonal line represent extinct plutonium haloes that would not now contain enough ^{244}Pu to distinguish them from thorium haloes.

Uranium 236 is formed in the decay chain of ^{244}Pu and has a half life of 2.4×10^7 yr. In transient equilibrium the ratio $^{236}\text{U}/^{244}\text{Pu}$ will be 0.41. Measurement of ^{236}U would suggest the presence of a plutonium halo.

Another distinctive feature of ^{244}Pu that might permit unambiguous identification of plutonium haloes is its relatively short half life for spontaneous fission. For ^{244}Pu the fission half life is 6.6×10^{10} yr (ref. 15), much less than the $\sim 10^{16}$ yr for ^{238}U , $\sim 10^{17}$ yr for ^{235}U and the $> 10^{21}$ yr for ^{232}Th (ref. 16). Consequently, for every 800 normal α -particle decays there will be a spontaneous fission producing two fragments.

The characteristic asymmetric mass and charge distributions of these fragments might serve to identify the halo as a plutonium halo. Of course, in such a distribution any given mass constitutes at most 7% of the total number of fragments, and if the level of detection by mass spectrometry is 10^6 atoms (about the best possible and it varies widely with the element), there will have to be a total of 10^7 fragments present to make the most abundant fragment isotopes detectable. Thus, about 4×10^9 atoms of ^{244}Pu is the smallest amount that will produce a detectable number of fission fragments. At least another factor of 10 is needed before quantitative measurements are reliable. Therefore, inclusions originally formed containing more than 4×10^{10} atoms of ^{244}Pu should contain enough remnant fission fragments to indicate the earlier presence of ^{244}Pu . The threshold for detection of fission fragments in an inclusion is indicated in Fig. 1 by the horizontal cross-hatched band.

Fission fragments have the advantage of indicating the nature of the halo whether the ^{244}Pu in the inclusion is extinct or not. Of course, the haloes may have been subject to the vagaries of physical and chemical action that remove fission fragments or in some other way vitiate their usefulness as identifiers. One of the most abundant fragments is xenon, but it is especially easily removed because it is a gas.

Anomalous large haloes

The fission of ^{244}Pu could lead to the production of haloes larger than those commonly observed. In about one out of 400 fissions an energetic α particle may be expected to be emitted¹⁶. In the spontaneous fission of ^{252}Cf , in the induced fission of ^{240}Pu and in all other cases of fission studied these α particles have a roughly Gaussian distribution of energies from 0 to 30 MeV with a peak around 16 MeV (refs 16–19). Because about 0.6 of the emitted α particles have energy greater than 15 MeV and a 15-MeV α particle has a range of $\sim 100 \mu\text{m}$ in biotite, it would seem possible to generate haloes as large as 100 μm or even larger, certainly much bigger than the normal size of 40 μm .

The production of large plutonium haloes, however, is by no means as probable as the production of normal plutonium haloes. To generate a number of fission alphas to produce a fluence of $\sim 10^{13}$ α particles per cm^2 at a distance of 100 μm from the surface of an inclusion requires an inclusion so large that self-absorption becomes a serious problem.

A simple model illustrates the difficulty. Consider a 100- μm diameter spherical inclusion of a pure crystal of 'plutonite', a hypothetical mineral of plutonium phosphate of density 7.5 g cm^{-3} and molecular weight 339. The Bragg–Kleeman rule predicts that a 15-MeV α particle will have a range of 79 μm in this mineral. Self-absorption in the inclusion will be important.

A simple numerical integration of this model taking self-absorption into account and assuming that the emitted fluence of α particles has a Gaussian energy distribution with a peak at 16.4 MeV and FWHM = 10.8 MeV, yields a fluence of 10^{13} α particles per cm^2 at 50 μm from the surface and 1.9×10^{12} α particles per cm^2 at 100 μm . Both of these numbers are smaller than the 4×10^{13} α particles per cm^2 assumed above as necessary to initiate coloration of a halo. Making the inclusion larger or smaller does not eliminate the problem.

Nevertheless, the possibility of anomalously large haloes still needs consideration chiefly because the threshold of coloration is poorly defined. Our knowledge of the threshold is based on experiments which assumed reciprocity between intensity and duration of exposure and used much higher rates of exposure than occur around haloes in nature. Yet reciprocity is known to fail in the coloration processes⁶.

The possible failure of reciprocity is interesting in two respects. Unlike normal haloes which are terminated by the limits of the ranges of the alphas emitted from the inclusion, haloes produced by α particles from spontaneous fission would be limited by the inverse square law. Consequently, the volume 'outside' the halo would be subject to an appreciable α -particle fluence over periods of geological time. There have been no laboratory investigations of circumstances like these and it is conceivable that coloration might occur at fluences much below the nominal threshold.

We recognise that these inclusions and their immediate environment would be subject to strenuous conditions very different from those under which the laboratory determinations of threshold were made. The plutonium mineral would in the course of the lifetime of a halo convert itself into a thorium mineral. Also inside and around the inclusion there would be a large fluence of α particles from the normal mode of decay. The inclusion in the model described above would contain 7×10^{15} atoms of ^{244}Pu which would emit 21×10^{15} α particles with energies from 4.5 to 5.2 MeV. This is a fluence of 5×10^{20} α particles per cm^2 at 18 μm from the surface. These α particles would deposit more than 15,000 J in and around the crystal, which would consequently be metamictised, undergoing changes of density and varying degrees of recrystallisation. The amount of helium generated would be large, as can be appreciated by realising that if it did not diffuse away it would produce a pressure more than 600 atm at room temperature in the 68- μm radius volume of the inclusion and halo.

Furthermore, there would be 14×10^{15} electrons emitted

from the two stages of beta decay in the normal decay chain of ^{241}Pu . With energies from 0 to 2 MeV these electrons would extend several hundred μm out from the inclusion and would deposit about 500 J in the spherical volume 150 μm in radius centred on the inclusion. Although this is about 1,000 times more energy per unit volume than is deposited by a threshold fluences of 4×10^{13} α particles per cm^2 , it is unlikely that the β particles produce coloration by themselves because there is no evidence that similar intensities of β rays outside large inclusions rich in Th or U have produced coloration⁶. A large inclusion rich in ^{241}Pu , however, has a unique combination of an appreciable α particle fluence along with a large β -particle fluence. It is possible that the β particles might activate the mica and lower the coloration threshold for the α particles.

The foregoing considerations suggest that a variation in the threshold for coloration by large amounts from values based on laboratory experiments is definitely possible.

Giant haloes

There is an important reason for pressing to its limits the possibility of ^{241}Pu as a source of large haloes. Such haloes have been observed, but no adequate explanation of them exists. Figure 2 shows the relationship between distribution of haloes and their size, as observed by Gentry¹⁰. The sharp peak at 37–43 μm is attributed to normal uranium and thorium haloes. The peak at 50–58 μm has been attributed to the 10.6-MeV α particle from $^{212}\text{Po}^*$ which has a branching ratio of 1:5,500 in the usual thorium decay chain¹⁰. (As can be seen, this peak appears too high for such a small branching ratio.) The larger haloes are unexplained.

The possibility of producing large haloes depends on the ratio of the partial half life T_1 for emitting long-range α particles during spontaneous fission to the overall half life T_0 of the nuclide. It is helpful to relate this ratio to our model mineral inclusion and the nominal threshold fluence ϕ required to produce a halo out to a distance r_0 from the surface of the assumed spherical inclusion of density ρ , molecular weight A , and radius r . A useful inequality between T_1 and T_0 follows from the fact that the ratio of the number of radioactive nuclei in the inclusion to the number of long-range α particles passing through a surface a distance r_0 from the inclusion must be greater than or equal to the ratio T_1/T_0 , that is

$$(4/3\pi r^3 \rho N_a)/(4\pi(r+r_0)^2 \phi) \quad R \geq T_1/T_0$$

where N_a is the Avogadro constant. The 'registration factor' R has been introduced to take into account enhanced ionising efficiency, absorptive losses, different concentrations of plutonium, or deviations of actual from nominal threshold. Let $a_0 = (r+r_0)/r$, then

$$T_1 a_0^2 (a_0 - 1)/R \leq T_0 \rho N_a r_0 / (3A\phi)$$

is an inequality which must be satisfied in order to produce giant haloes by α particles accompanying fission.

In terms of our hypothetical plutonite inclusion with $r = 50 \mu\text{m}$, $r_0 = 100 \mu\text{m}$ and a threshold fluence of 4×10^{13}

$$T_1/18R \leq 1.1 \times 10^6 T_0$$

For plutonium $T_1/T_0 = 32 \times 10^4$, so in 'plutonite' a registration factor of 5.2 is needed to satisfy the inequality. The numerical calculation referred to above gives $R = 0.25$. Thus to meet the conditions of the inequality, the registration factor must be increased by a factor of about 20.

It is easy although not altogether convincing to increase R . The uncertainty in the threshold is probably a factor of at least 40. It is only necessary to believe that a fluence of 10^{12} α particles

per cm^2 over geological time would produce colour in a region adjacent to one undergoing coloration and in the presence of a large fluence of electrons. In the absence of any information about the mechanism of colour formation in haloes, this is at least a possible assumption. In addition, the peak in the Bragg ionisation curve folded into the inverse square decrease in ionisation is, at the end of a 100- μm track, 30% higher than at the minimum on the track. With about half the nominal fluence it would be possible to achieve coloration at the ends of tracks although some intermediate parts along the tracks would not be coloured. Or again, metamictisation or recrystallisation might lower the density sufficiently to increase the range by 20% and thus the fluence at r_0 by as much as 40%. Together these factors could produce an R of 16.

It is necessary to recognise that only pure 'plutonite' has been discussed here. Most mineral inclusions are not pure. Mixtures of plutonite with other substances to lower the registration factor by factors of two, three or more are likely. This dilution of plutonium might have been offset by preferential fractionation of plutonium such as was mentioned above⁵. It is therefore appropriate to modify our model inclusion by assuming that it is only 50% plutonite. Under this assumption the registration factor for the model inclusion will be $R = 0.13$; optimally, for this model, R might equal 8.

Therefore, it seems likely there could be haloes of 50–60 μm size, and it seems conceivable that optimum conditions could result occasionally in giant haloes of up to 100 μm around an inclusion containing ^{241}Pu . Such a sequence of probabilities is consistent with the distribution of halo sizes shown in Fig. 2.

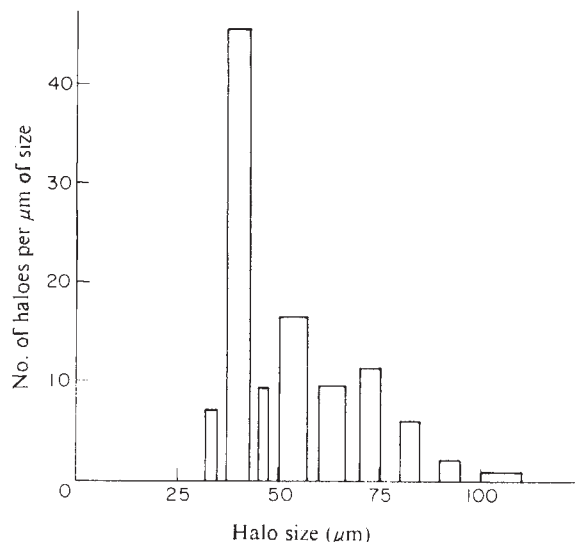


Fig. 2 Distribution of haloes by size. Size is measured from the nearest surface of the inclusion to the outer edge of the halo. These data are adapted from ref. 10.

It should be quite straightforward to determine whether a giant halo is due to ^{241}Pu . The size of the halo will be directly related to the size of the inclusion. The largest haloes should occur only around inclusions with dimensions of 50–100 μm ; the smaller the inclusion, the smaller the halo. The short half-life of ^{241}Pu suggests that the substantial concentration of ^{241}Pu required to make a giant halo would have been more likely long ago, so the rock containing giant haloes should be old. Giant plutonium haloes should also have quantities of fission fragments in and around the central inclusion which could easily be detected by mass spectrometry and even by X-ray spectrometry. (Our model plutonite inclusion and halo

would contain about 10^{13} fragments.) The fragments are 800 times as abundant as the α particles emitted in fission. There might also be detectable amounts of ^{241}Pu in the inclusion, but if the minimum number of nuclei needed to produce a fission α particle halo is 10^{15} , Fig. 1 shows that the rocks in which such haloes occur must be younger than 2.5×10^9 yr if plutonium is to be detectable.

Because of the vigorous thermal, physical and chemical history of the inclusion, it is likely that most etchable fission tracks in the vicinity of the inclusion would have been annealed out.

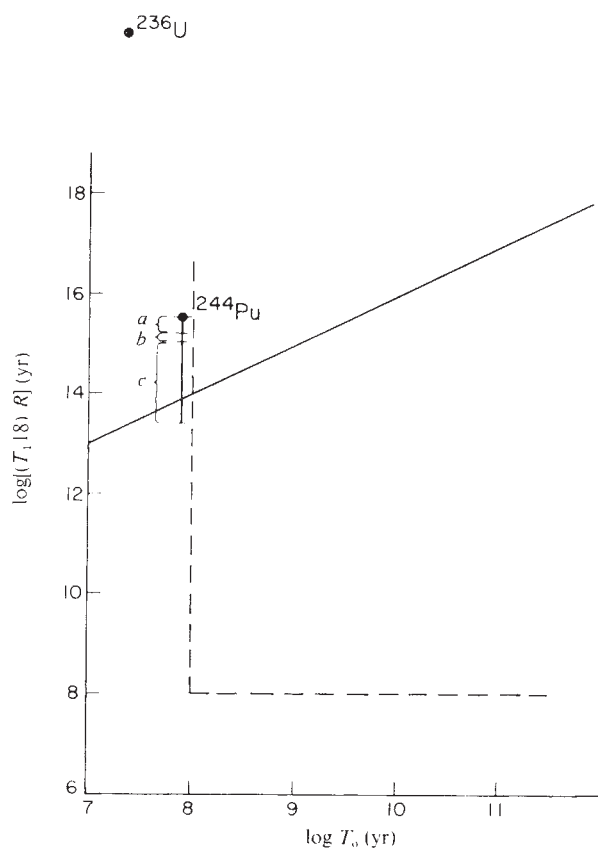


Fig. 3 Relation of α particle-fission half lives and overall half-lives that will permit production of giant haloes by fission alphas. The diagonal line is drawn assuming a threshold fluence of 4×10^{13} α particles cm^{-2} . Possible corrections to the location of ^{244}Pu are *a*, Bragg ionisation, *b*, changes in density and range, and *c*, lowering of the coloration threshold. At present no isotopes are known with values in the preferred region below the diagonal line and above and to the right of the dashed lines. ^{244}Pu is the closest.

The graph in Fig. 3 plots $T_1 a_0^2(a_0 - 1)/R$ against T_0 . (R is a function of a_0 , so a_0 cannot be varied independently of R .) For a nuclide to produce giant haloes by fission α particles, its half lives must define a point in the region below the diagonal line defined by the inequality, and above and to the right of lines corresponding to 10^8 yr. These last constraints are not well defined but take into account that if its half life were much shorter than the age of the Earth the likelihood of a nuclide being around long enough to be incorporated into a mineral is small. Plutonium 244 probably represents a lower limit.

Superheavy elements

The possibility of giant haloes from fission α particles is relevant to the suggestion of Gentry *et al.*¹¹ of the existence of the

elements $Z = 126, 127, 116$ and 124 , based on the X-ray spectra produced by proton bombardment of the inclusions of several giant haloes. The analysis of their spectra is complicated by the necessity of subtracting a large background from a nearly equal foreground. It seems possible that they may have been studying plutonium haloes and inclusions rich in fission fragments. The very elements they must argue are not contributing to the peaks attributed to superheavy elements are ones expected to be among fission fragments: Te, In, Sb and Xe. Moreover, the intensity of the peak identified as the $L_{\alpha 1}$ of $Z = 126$ is roughly of the right size and energy to be K_{α} X rays from $\sim 10^{11}$ Te fission fragments, somewhat less than the number to be expected in our model sample.

The association of the peak with $Z = 126$ is most strongly supported by the apparent discrepancy of 173 eV between the observed position of the peak (which is about three channels and 350 eV wide) and the expected position of the centroid of the Te K_{α} lines. The background, however, is so large that it seems possible that the subtraction procedure might shift the apparent position of the centroid enough to account for the difference.

The uncomfortable stretching of parameters required to form a ^{244}Pu giant halo is not the only alternative to balance against the hypothesis that the superheavy elements exist in their inclusions in quantities of "several hundred picograms" (ref. 11). There is the alternative of attributing giant haloes to an as yet undiscovered isotope or its short-lived descendants. The isotope could of course be a superheavy, but it seems as plausible that a new isotope could be a member of a known atomic species with a half life of $\sim 10^9$ yr and either a fission half life not much greater or a daughter isotope with a short fission half life.

Conclusions

It is reasonable to expect to find in nature pleochroic haloes produced by extinct or nearly extinct minerals of ^{244}Pu . These could be identified visually, by vestigial ^{244}Pu or ^{236}U in the central inclusion, or by the presence of the appropriate fission fragments.

Larger than normal haloes, perhaps up to $100 \mu\text{m}$, might arise from the energetic alpha particles that occasionally accompany the spontaneous fission of ^{244}Pu . If the giant haloes observed in nature are not produced by the fission α particles of ^{244}Pu , they might, as Gentry *et al.*¹¹ suggest, come from elements $Z = 126, 127, 116$ and 124 , or they may be caused by the fission α particles of some as yet undiscovered isotope.

To determine the relative probabilities of these phenomena, further study of the chemistry of plutonium pertinent to geological processes and mineral formation is required. Also, the coloration process should be examined, especially in the presence of combinations of fluxes of different radiations.

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Tomato bushy stunt virus at 5.5-Å resolution

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The coat of tomato bushy stunt virus is built from protein subunits having rigid domains connected by a flexible hinge. Two states of the hinge are present in the $T = 3$ icosahedral structure. Each subunit has a binding site for RNA on its inner surface.

DESIGN principles of regular viruses were first clarified by Caspar and Klug¹ who showed that quasi-equivalence could reconcile the variability of subunit packing (required to construct a shell of sufficient size) with the specificity of subunit bonding, necessary for self-assembly. The small, spherical RNA viruses pose two important structural questions: how is quasi-equivalent packing of identical protein subunits achieved, and how is the nucleic-acid chain accommodated in the particle? We describe here a 5.5-Å resolution electron-density map of tomato bushy stunt virus (TBSV), and derive some initial answers.

The TBSV particle comprises a single molecule of RNA (MW 1.5×10^6), 180 coat protein subunits (MW 41,000 each) and perhaps one copy of a larger polypeptide²⁻⁴. The coat protein forms a $T = 3$ icosahedral surface lattice, with prominent dimer clustering at the outside of the particle⁵. This design implies that a single kind of polypeptide chain packs in relation to its neighbours in three slightly different ways (Fig. 1). X-ray crystallography^{6,7} has revealed the orientation of local symmetry axes and suggested that the differences between quasi-equivalent contacts is related to RNA-induced perturbations.

The structure has been solved using multiple isomorphous replacement, combined with phase refinement by icosahedral averaging. Problems for data collection due to the large unit cell were solved using a focusing X-ray camera⁸, and a strategy for oscillation photography appropriate to the restriction that only one exposure can be obtained per crystal⁹. As in the 5-Å resolution study of the tobacco mosaic virus disk¹⁰, a problem of comparable magnitude, exploitation of the non-crystallographic symmetry¹¹ has been important for obtaining a good map.

Data collection and phase determination

TBSV crystals, obtained as described before⁷, are cubic, space group 123, $a = 383$ Å. Each lattice point of the body-centred cubic cell is occupied by one virus particle, such that the crystallographic point group is the tetrahedral subgroup of the viral icosahedral symmetry.

X-ray intensities were measured by oscillation photography from crystals of the native virus and of two derivatives, PtCl_6^{2-} and Hg^{2+} (Table 1). Coordinates for the PtCl_6^{2-} derivative were available from earlier work, refined in projection to 9 Å (ref. 7). A difference-Fourier $hk0$ -projection for the Hg^{2+} derivative at 9 Å, (from a 6° precession photograph), using signs from PtCl_6^{2-} , revealed two sets of quasi-equivalent sites (six sites per icosahedral asymmetric unit). One of these corresponds to the CH_3Hg^+ sites used in the 16-Å work⁷, and the other to positions near the subunit tips. After conventional refinement, the average figure-of-merit was 0.57 (Table 2). Fivefold molecular averaging was then carried out using recently developed methods¹¹. After one cycle of phase refinement the mean figure-of-merit was 0.76 (Table 2). Previous

experience suggested that additional cycles were unlikely to improve the map further^{7,10}. A second map was computed using the combined phases, icosahedrally averaged¹¹.

Electron density map

It is convenient to look at the electron density map in sections normal to a crystallographic twofold (arbitrarily chosen as the Z -axis). The 16-Å resolution map⁷ shows that the quasi-threefold axis is approximately parallel to the nearest strict twofold (Fig. 1), so that quasi-threefold related electron density features appear in almost the same Z -sections. The triangle shown in Fig. 1 is thus a useful choice for the icosahedral asymmetric unit, and we chose to contour on acetate sheets a region somewhat larger than this triangle, indicated by the rectangular outline.

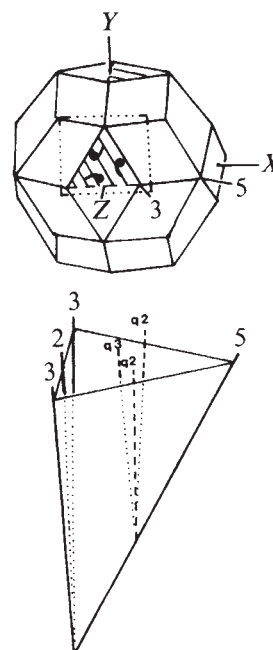


Fig. 1 Orientation of TBSV symmetry axes and choice of icosahedral asymmetric unit. *a*, Rhombic triacontahedron, showing some icosahedral symmetry axes. This figure has icosahedral symmetry, and it is useful for representing a $T = 3$ icosahedral surface lattice. The triangular half-face (shaded) is an asymmetric unit of the surface, that is, this region, replicated by the various symmetry operations, will cover the entire surface. In a $T = 3$ lattice, there is a local threefold axis near the centre of this triangle, relating the three quasi-equivalent subunits in the icosahedral asymmetric unit. Note that X , Y and Z are twofold axes. The rectangle indicates the area of the electron-density map, computed in sections normal to Z , chosen for contouring (compare Fig. 2). *b*, An asymmetric unit of volume in a region governed by icosahedral symmetry. This volume, replicated by the various symmetry operations, will fill the rhombic triacontahedron in (*a*); it can be thought of as the domain swept out by the upper shaded triangular half-face in (*a*) on a series of concentric rhombic triacontahedra of continuously increasing radius. Orientations of local (quasi)symmetry axes (denoted by small numbers) are also shown. The quasi-twofold and threefold axes around each fivefold axis intersect it about 60 Å out from the particle centre.

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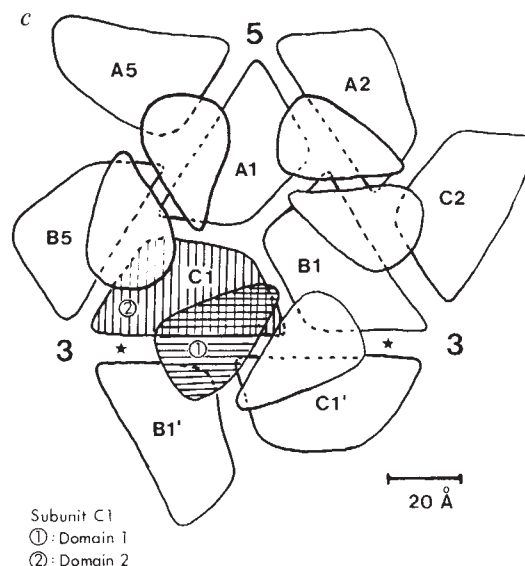
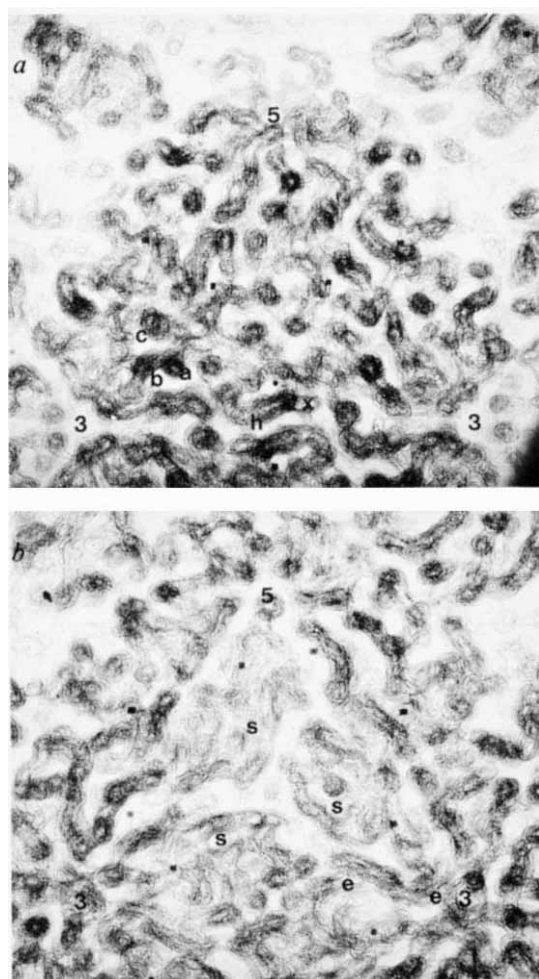


Fig. 2 Views along Z through sections of the TBSV 5.5-Å electron density map. *a*, $Z = 125-140$ Å; *b*, $Z = 110-125$ Å. Figure 1 gives orientation. Some features of secondary structure are indicated by lower-case letters: X is the inter-domain connection; h , the tangential rod; a , b and c , some radial rods; s , the saddle-like feature; and e , the extra density. The subunit boundaries may be judged by reference to (*c*). *c*, Packing of subunits and domains. A1, B1 and C1 represent subunits packed in one icosahedral asymmetric unit, related to each other by quasi-threefold rotation; A2, ..., A5, are related to A1 by fivefold rotations; similarly, B1, ..., B5 and C1, ..., C5; B1' is related to B1 by a strict dyad, A2 to B1 and B5 to A1, by quasi-dyads. The domains are indicated in rough outline: barrel-shaped domains 1 protrude outwards from the surface, making only twofold contacts; the more extensive domains 2 make contacts across all adjacent symmetry axes, forming a continuous polyhedral shell. The shading of C1 indicates which domain 1 is associated with the labelled domain 2. Domains 1 for C2, B1' and A5 are not shown. Stars indicate the interrupted contact ('cleft'), occupied by RNA. The scale is the same for (*a*), (*b*) and (*c*).

The electron-density features within this icosahedral asymmetric unit begin at about $Z = 112$ Å and extend out to $Z = 170$ Å. At $Z \sim 110$ Å there are faint and rather wispy but relatively continuous features. From $Z = 112-140$ Å, the volume is entirely filled with dense features, and at larger values of Z , density is concentrated more and more around the strict and quasi dyads, leaving large gaps in between. The icosahedrally-ordered part of the virus structure can thus be described as a roughly 30-Å thick, compact polyhedral shell, from which 90 protrusions, the dimer-clustered morphological units so clearly seen in electron micrographs, emerge.

Boundaries and domains

Figure 2*a* shows the composite electron density of sections from the central part of the polyhedral shell. Local threefold symmetry is visible in the central part of the asymmetric unit, and there is striking similarity of corresponding parts of quasi-equivalent subunits. Assignment of subunit boundaries was aided by the requirement that symmetry-related density features near an axis must belong to different subunits, even though they may seem weakly connected. A good illustration of the clarity of most boundaries is the gap running more or less along the lower edge of the icosahedral asymmetric unit (connecting the strict threefolds) in Fig. 2*a*. The quasi-equivalent boundaries, not as pronounced in this view, show up equally well if viewed along the local dyad.

The most outstanding characteristic of the subunit is that it falls into two very distinct, globular domains (Fig. 3). The smaller of these (domain 1) forms half of one of the dimer-clustered protrusions; the larger (domain 2) forms part of the polyhedral shell. Domain 2 has a rather distinct, saddle-like feature just against its innermost surface, which we describe in the next section. The two domains are connected at only one place by a narrow

Table 1 Statistics of data collection to 5.5 Å

	Native	PtCl ₆ ²⁻	Hg ²⁺
Total fully recorded reflections measured	69,000	75,000	65,000
No. of independent reflections measured	26,700	27,100	26,700
$R_{\text{sym}} = \sum_{h,k,l} I_i - \bar{I} / \sum_h \bar{I}$	0.100	0.133	0.145
Mean isomorphous difference $= \langle I_{\text{nat}} - I_{\text{der}} \rangle / \langle I_{\text{der}} \rangle$	—	0.29	0.56

Data were collected by oscillation about [101], the shortest axis in reciprocal space; rotation from 0° to 20° (where 0° corresponds to [101] coincident with the X-ray beam) includes more than 90% of the 29,000 independent reflections to 5.5 Å. The crystals thus always present a rather plate-like aspect to the X-ray beam, which is much smaller than the crystal diameter, so absorption effects are minimised. The oscillation range of a single 20–24-h exposure was 55–65° of arc. Exposure usually exhausted the crystal lifetime, so that we could not add partially recorded reflections from abutting photographs¹². Partial reflections, about $\frac{1}{3}$ of those recorded, were not included in the data set but the ratios of the measured intensities of some of them to the intensities of corresponding symmetry-related whole spots on the same film, were used to obtain an accurate estimate of crystal orientation and hence an accurate discrimination between fully and partially recorded reflections⁹. Because of the small cross-fire in the focused beam and the small mosaic spread of the crystals, a 10–15° overlap of adjacent exposures ensured that all reflections were fully recorded on film. Films were densitomered using a PDP-11/20-linked Optronics Photoscan and a modified version of the program SCAN 12 (ref. 13). The output included an integrated intensity and a standard deviation for each reflection, the latter determined from the actual standard deviation of the measured background. The three films in a pack were scaled to each other by the method of Fox and Holmes¹⁴. The approximately 3,000 fully-recorded reflections were sufficient to generate a Wilson-type plot of $\langle I \rangle$ against $\sin^2 \theta / \lambda^2$, and these plots were used to determine relative scale and temperature factors for each film pack.

Table 2 Phase determination

	Site	Z	B	Heavy-atom parameters		
				x	y	z
PtCl ₆ ²⁻	A	55	53	-0.0157	0.1493	0.3060
	B	49	46	0.0756	0.0481	0.3014
	C	53	48	-0.0619	0.0203	0.3001
	D	19	35	-0.1132	0.0059	0.2933
$R_c = 0.58$, $R = 0.56$, r.m.s. f_c /r.m.s. $E = 1.86$, mean ΔF /mean $F = 0.12$						
Hg ²⁺	A1	80	25	-0.0372	0.0905	0.3339
	B1	71	27	0.0358	0.0923	0.3344
	C1	81	35	0.0010	0.0271	0.3360
	A2	71	25	-0.0680	0.1205	0.4234
	B2	66	28	0.0822	0.0976	0.4277
	C2	76	25	-0.0146	0.0145	0.4301
$R_c = 0.55$, $R = 0.53$, r.m.s. f_c /r.m.s. $E = 1.9$, mean ΔF /mean $F = 0.26$						

Z, occupancy in electrons, on experimental absolute scale⁷; B, heavy-atom 'temperature factor'; R, $\Sigma||\Delta F_{\text{obs}}| - |\Delta F_{\text{calc}}||/\Sigma|\Delta F_{\text{obs}}|$, all reflections; R_c (Cullis R factor), R for centric reflections; E, lack of closure at best phase; f_c , heavy-atom structure factor; r.m.s., root-mean square.

Statistics of molecular averaging up phase refinement.

Figures of merit: initial (DIR), 0.57; final (combined), 0.76.

Comparison of calculated and observed structure factors:

$$R = \langle ||F_{\text{obs}}| - |F_{\text{calc}}|| \rangle / \langle |F_{\text{obs}}| \rangle = 0.37.$$

$$\text{Mean phase difference} = \langle |\alpha_{\text{calc}} - \alpha_{\text{DIR}}| \rangle = 47^\circ.$$

$$\text{Mean phase change after combination} = \langle |\alpha_{\text{final}} - \alpha_{\text{DIR}}| \rangle = 25^\circ.$$

Alternate rounds of phasing and icosahedrally-constrained least-squares refinement^{7,15} used starting coordinates obtained as described in the text. Coordinates and occupancies for both derivatives were refined in projection, first at 9 Å then at 5.5 Å, and finally in three dimensions against a set of approximately 3,500 reflections representing a random sample of resolution and intensity ranges, selected with the criterion that the precision of heavy-atom intensity differences meet a pre-determined minimum. After refinement in projection, self and cross-difference maps gave no evidence for additional sites, a conclusion confirmed in three dimensions. Site names correspond to the subunit to which they were subsequently seen to bind (compare Fig. 2c), and only one icosahedral asymmetric unit of sites is given. The quoted values of R_c and so on, refer to the 3,500 well-measured reflections used for the refinement. For icosahedral phase refinement, an initial map, computed on a 1.1-Å grid for accurate interpolation¹¹, was averaged about the icosahedral fivefold axes to determine the molecular envelope; density not part of the particle to which these axes refer is smeared out by this procedure, enhancing the boundary contrast. The final envelope for an entire virus particle is a rhombic triacontahedron (Fig. 1) with roughly cylindrical 'pillars' at each twofold and quasi-twofold position, where subunit tips protrude⁷. Averaging and setting all density outside this envelope to zero yielded a symmetrised map (2.2-Å grid), from which structure factors were computed. The agreement between observed and calculated amplitudes is tabulated: most of this residual comes from the scatter of the figure-of-merit around its mean value in resolution shells¹¹. Calculated and DIR phases were combined using the Sim formula¹⁶.

region of strong electron density: each domain thus appears to contain one terminus of the polypeptide chain.

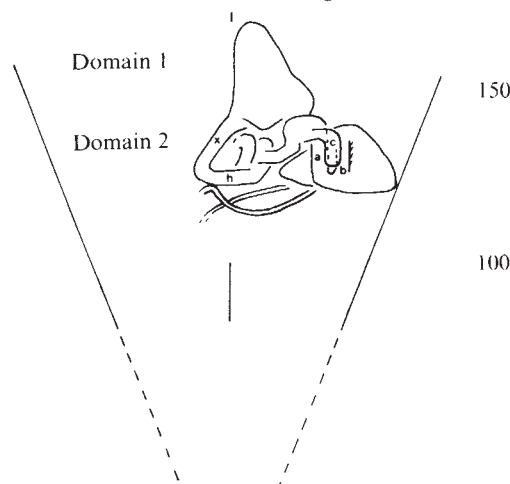
Domain 1 is about $30 \times 30 \times 25$ Å in overall dimensions. It consists of two approximately parallel layers, oriented such that one layer forms an extended and tight dimer contact with domain 1 of the twofold (or quasi-twofold) related subunit. The general distribution of electron density in this domain resembles very strongly the β -barrel structures, also found dimer-clustered, in immunoglobulin fragments^{17,18} and in superoxide dismutase¹⁹. The outer layer of the barrel is markedly curved; the inner appears quite flat, especially in the contact region. The strand directions in each layer cannot be established with certainty in a 5.5-Å map, but there is definite indication of a radial rather than tangential course. The outer layer of the barrel is markedly curved; the inner seems quite flat, especially in the contact region. The strand directions in described below.

The connection of domains 1 and 2 is well defined and occurs at the point marked with a cross in Figs 2a and 3. Domain 2 itself is considerably more complex than domain 1, both in its internal structure and in its interactions with adjacent subunits. Nevertheless, its boundaries appear rather well determined, with one major ambiguity (see below). In tracing a boundary, we used the symmetry criteria described earlier and assumed that the domain is a reasonably compact globular unit. Figure 2c shows schematically the relation of domains 1 and 2 and the nearest-neighbour packing in the protein shell.

Domain 2 has a number of rodlike features indicative of α -helices (these are absent in domain 1). These rods are oriented more or less radially with one important exception, which we refer to as the tangential rod (h, in Fig. 2a). As seen in Figs 2a and 3, one end of this rod connects with the feature that forms the junction between the two domains. The tangential rod is 15 Å long (2–3 turns of α -helix) and located close to the dyad, such that the distance between twofold-related helical axes is 10 Å. Each rod is tilted slightly out of the plane normal to the dyad, and we have chosen the enantiomorph such that the tilted rods have a left-handed relationship. This packing is appropriate for right-handed α -helices^{10,21}. Closely-packed, radially-oriented rods in domain 2 show the same relative tilt, and the twist of the outer wall in the

domain 1 β -barrel is also consistent with this choice. The three most prominent radially-directed rods (a,b,c in Fig. 2a) seem to be in direct contact. Their lengths correspond to 2–3 turns. Rods a and b are tightly packed, whereas rod c is somewhat farther away. The principal ambiguity in boundary tracing, mentioned above, concerns the assignment of c and some connected density to subunit C1 (Fig. 2c). The alternative assignment of c to A1 leads to

Fig. 3 Schematic representation of the electron density in one subunit, viewed normal to the adjacent dyad and looking toward that dyad from the quasi-threefold. If the drawing is taken to represent C1 in Fig. 2c, then the direction of view is downwards in the plane of that figure. The lobe at the outside of the subunit ($Z = 140$ – 170 Å) is the β -barrel of domain 1; the cross indicates its connection to the more extensively α -helical domain 2; the 'saddle' lies beneath domain 2, and a tentative interpretation in terms of two stretches of RNA backbone is shown in the diagram.



a more extended domain 2, but the choice is arbitrary. In the pentamer (or hexamer) contact regions near the bottom of domain 2, the density has a layer-like character, suggestive of β -sheet. Rods *a* and *b* are packed partly against this sheet-like feature. There are a number of shorter pieces of rodlike density, less clearly α -helices. One of these occurs close to the local threefold axis: the three such symmetry-related rods appear to form a sort of rudimentary, left-handed, three-stranded coiled-coil²¹.

We estimated the volume occupied by all the strong features of one subunit as $51,000 \pm 6,000 \text{ \AA}^3$, where the upper and lower limits correspond to generous and restricted estimates of surrounding side chain, Fourier cutoff and so on. If all this volume were protein, the corresponding MW would be $39,000 \pm 5,000$ (taking $r = 0.74 \text{ cm}^3 \text{ g}^{-1}$). Twelve nucleotides, our upper estimate for RNA in the saddle feature, occupy about $3,400 \text{ \AA}^3$ (taking $r = 0.51 \text{ cm}^3 \text{ g}^{-1}$). We can thus account for most of the protein subunit, although some 10% of the chain could be outside strong density in the present map.

Nucleic acid

A significant problem in interpreting the TBSV electron-density map has been to establish criteria for recognising RNA. Strength of the density alone proved insufficient: the features described below are among the strongest in the map but no stronger than the α -helices. This is not surprising because partial occupancy of polynucleotide binding sites and other sorts of static disorder would diminish the corresponding density. A general picture of the nucleic acid might be one of ordered regions of backbone, bound to protein, interspersed with more disordered regions at smaller radii. The overall appearance of the map at about $110 \text{ \AA}$ from the particle centre is consistent with this view: strong features trail off towards the centre in little streamers of density. The distribution of this streaky density suggests that the polynucleotide chain may enter or leave a binding region in more than one way, but the strength of the beaded features to which they join indicates a definite conformation in the binding site.

A striking aspect of the map is the complicated and cross-connected texture of features between 110 and $120 \text{ \AA}$, in contrast to the rather clear sheet-like or rod-like density of domains 1 and 2. In this region, each icosahedral asymmetric unit shows three very similar, though not precisely identical, saddle-shaped features, about $30 \times 20 \times 10 \text{ \AA}$ in extent (s in Fig. 2*b*). Each of these saddles forms the innermost density associated with one protein subunit. In addition, there is a set of strong features with no quasi-equivalent counterpart extending from near the strict twofold toward the strict threefold axes; we refer to this as extra density (e in Fig. 2*b*). Since the protein subunits are identical and since RNA is the only other significant component, this extra density is likely to represent part of the nucleic acid chain. Inspection of a $5.5\text{-\AA}$ resolution map of yeast-phenylalanyl tRNA (computed as an F_{calc} map by A. Jack, MRC Laboratory of Molecular Biology, Cambridge) shows that the sugar phosphate backbone has a quite characteristic appearance: a continuous stretch of density with barely resolved maxima spaced at distances of $6\text{--}7 \text{ \AA}$, at or near the positions of phosphates, giving it a beaded aspect. Both the extra density and certain parts of the edges of the saddles have just this $6\text{--}7\text{-\AA}$ beading. Moreover, the saddle lying beneath subunit C is connected to the extra density at several points, with the $6\text{--}7\text{-\AA}$ periodicity continuing from one to the other. We therefore conclude that a significant part of the density between 110 and $120 \text{ \AA}$ represents polynucleotide chain.

The saddle features form rather distinct lobes of density just against the bottom of domain 2. We followed possible RNA backbone configurations in this region, although, in view of the ambiguities involved, such a tracing is rather speculative at this resolution. We believe that we can recognise two pieces of RNA backbone (Fig. 3). One of these emerges from the particle interior and passes near the strict or quasi-twofold; the second forms the outer rim of the saddle. We detected 10–12 possible phosphates. Density between these chains could in part be nucleotide bases, and the backbones are so oriented that they could be joined by two or three base pairs in the A-RNA geometry.

No simple description would seem to summarise protein-RNA contacts. The structure of domain 2 shows that several portions of the polypeptide chain, separated in the amino acid sequence, must come together to form the shallow pocket in which the saddle feature lies. Stretches of α -helix, as well as a region that may be β -sheet, are involved.

States of the protein subunit

The clarity of the electron density map has facilitated quantitative analysis of subunit similarities. The coat protein assumes two clearly different conformational states, depending on whether it is near a local or a true dyad in the $T = 3$ surface lattice. The individual domains 1 and 2 are essentially invariant, and a hinge between the rigid domains relates the two subunit conformations.

Corresponding well defined electron density maxima could easily be recognised in all three quasi-equivalent subunits and their superposition determined after appropriate rotation and translation. Referring to Fig. 2*c*, subunits A and B superpose very well, whereas A (or B) and C do not. Taken separately, however, domains 1 and 2 of A (or B) can be superposed with their analogues in C as well as A and B can be matched to each other. This implies that the relative orientation of domains 1 and 2 in C is different from their relative orientation in A or in B, but that individual domains are nearly invariant. The hinge between domains is

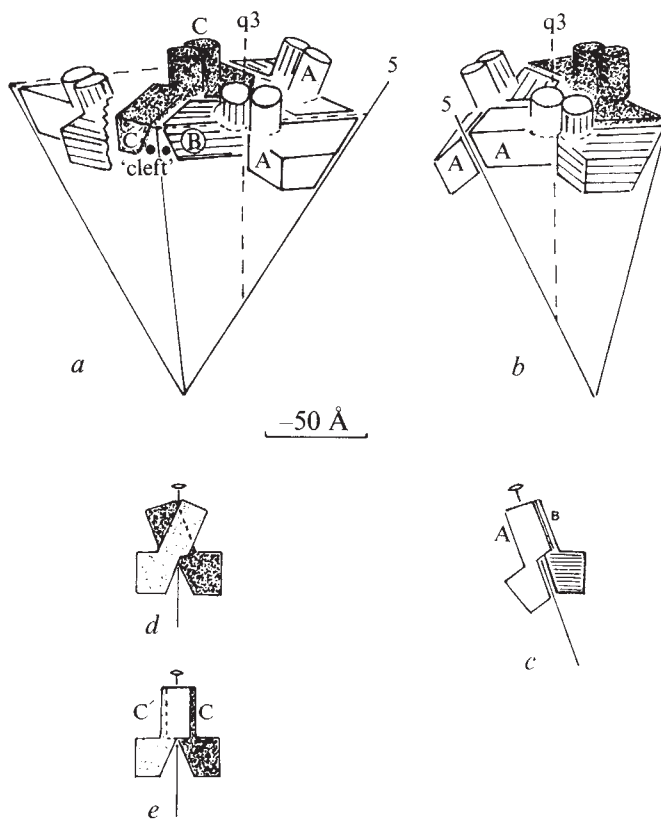


Fig. 4 Packing diagrams, showing subunits and relative orientation of the two domains. *a*, View similar to Fig. 1*b*, with a second icosahedral asymmetric unit replicated by the strict twofold axis. Each subunit is represented by a cylinder (domain 1) on a block-like base (domain 2). The three quasi-equivalent subunits are shown: white (A), hatched (B) and stippled (C). *b*, View of an icosahedral asymmetric unit, rotated 120° with respect to the view in (*a*). Considering just the domain 2 contacts, note that the A-A contact about the fivefold axis and the B-A contact across the quasi-dyad are quite tight, whereas the B-C' and C-C' contacts about the quasi-sixfold and strict twofold axes are opened up to produce a cleft. The difference in the angle of contact across quasi (A-B) and strict (C-C') dyads requires a change in the angle between domains 1 and 2 to preserve the dimer pairing of domains 1. This is shown in (*c-e*); the A-B contact (quasi-dyad) is depicted in (*c*). If the contact between the domains 2 were altered, without a relative reorientation of domains 1 and 2 in each subunit, the C-C' contact (strict dyad) would appear as in (*d*). In fact, there is rotation by approximately 20° about a hinge between domains 1 and 2, producing the sort of contact shown in (*e*).

located just above the end of the horizontal α -helix (Fig. 3). The hinge rotation axis is approximately parallel to the axis of the tangential rod, and the angle of rotation is about 20° (for C, relative to A or B). There are small, though significant, differences between A and B, but the hinge position is clearly the same for both.

The inter-subunit contacts of the hinged protein are represented in Figs 2c and 4, which show how the hinge leads to quasi-equivalent subunit bonding. Domains 1 have large and essentially identical contacts across both local and strict twofold axes. Domains 2 are likewise in extensive contact around the local threefold axis, which, for these domains, approximates well to an exact triad. Schematically, the polyhedral shell formed by all the domains 2 can be represented by 60 wedge-like trimers in continuous contact across local dyads but with only their outer edges in contact across strict dyads (Fig. 4). This produces a cleft connecting strict threefold (quasi-sixfold) axes. The quasi-sixfold relationship is thus very inexact: domains 2 of C1 and B5 in Fig. 2c are related essentially as A1 and A2, whereas the analogous contact between B1' and C1 (star in Fig. 2c) is largely broken by the cleft. This is in fact the only place where contacts are not basically the same for all three quasi-equivalent subunits. It is the hinge between domains 2 and 1 of subunit C that permits the cleft to open (Fig. 4e); without it, TBSV protein could form only a simple $T = 1$ icosahedral shell. Since 'extra' electron density features appear in the cleft, but not in the tight contacts between C1 and B5 or A1 and A2, RNA may play a significant role in regulating assembly of correct-sized, $T = 3$ particles. RNA-free, $T = 1$ structures have indeed been observed in studies of reassembly of the related turnip crinkle virus²³.

Domains and hinges

The generalisation that large polypeptide chains will fold into smaller, compact domains has gained considerable strength from the several proteins solved thus far with subunit molecular weights greater than 30,000 (refs 24–26). The two domains of TBSV protein have approximate molecular weights of 15,000 and 25,000; in the scheme of Levitt and Chothia²⁷ they seem to be members of class II ($\alpha\beta$) and III ($\alpha + \beta$), respectively.

The TBSV subunit is not the first example of a protein with flexibly-linked domains. The immunoglobulins are the classic case of such a structure¹⁷, and the relation between light chains in the Mcg Bence-Jones dimer¹⁸ is precisely analogous to the situation in TBSV: one of the chains in this dimer (the chain with a

'heavy-chain-like' configuration) bends at a hinge point, in order to conserve strong inter-molecular contacts. This type of structure could lead to controlled polymorphism in assembly.

The presence of RNA in the cleft at the C1/B1' interface seems to dictate the nature of inter-molecular contact and hence the choice of a $T = 1$ or $T = 3$ design. Since the angle of the contact is determined by the hinge angle, binding of ligands to residues in the hinge region, or at the domain 1/domain 2 interface, could likewise influence the mode of association. In general, a subunit with flexibly-connected rigid domains can be a useful element for controlled switching between distinct states of a macro-molecular assembly. Work on a 2.8-Å resolution map of TBSV is underway.

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letters to nature

Far infrared observations of IRC + 10216

THE detection of infrared radiation in the 1.2–20 μ m band from IRC + 10216 was first reported by Becklin *et al.*¹. Toombs *et al.*² determined its angular size by lunar occultations at 2.2, 3.5, 4.8 and 10 μ m, and deduced that the source is a late-type carbon star surrounded by a dust shell with two components: a bright, optically thick central component with a characteristic diameter and temperature of 0.4" and 600 K, respectively, and an outer component of diameter 2" and a temperature around 375 K. The longest-wavelength ground-based observations were made by Low, Rieke and Armstrong³ at 34 μ m, and the only other far infrared measurements are those of Campbell *et al.*⁴. We present here measurements of IRC + 10216 at 100 μ m made with the 91-cm telescope of the NASA G. P. Kuiper Airborne Infrared Observatory⁵.

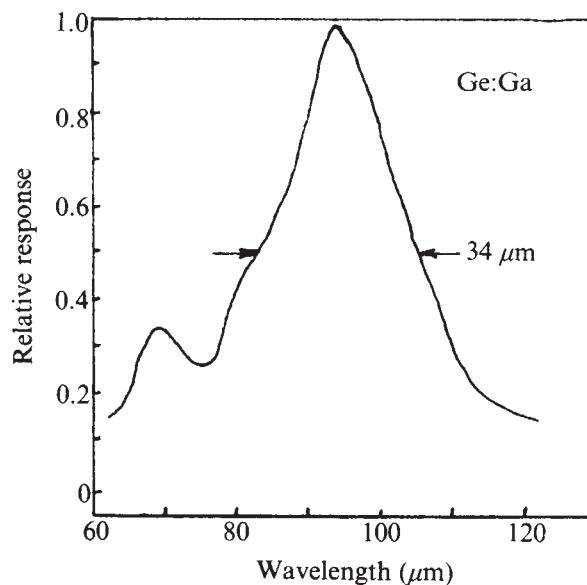
The infrared photometer consists of a gallium-doped ger-

manium (Ge:Ga) photoconductive detector with a Mosfet pre-amplifier and load resistor, all of which were cooled to liquid helium temperature. Fabry optics were used to reduce the background radiation on the detector, and a 4-mm aperture was used to define a 0.9' circular field of view. The instrumental passband was defined by cold filters (crystal quartz and Yoshinaga filters) at the field aperture. The spectral response of the detector-filter combination shown in Fig. 1a, was measured by using a Perkin-Elmer grating spectrometer and normalised to a Golay detector.

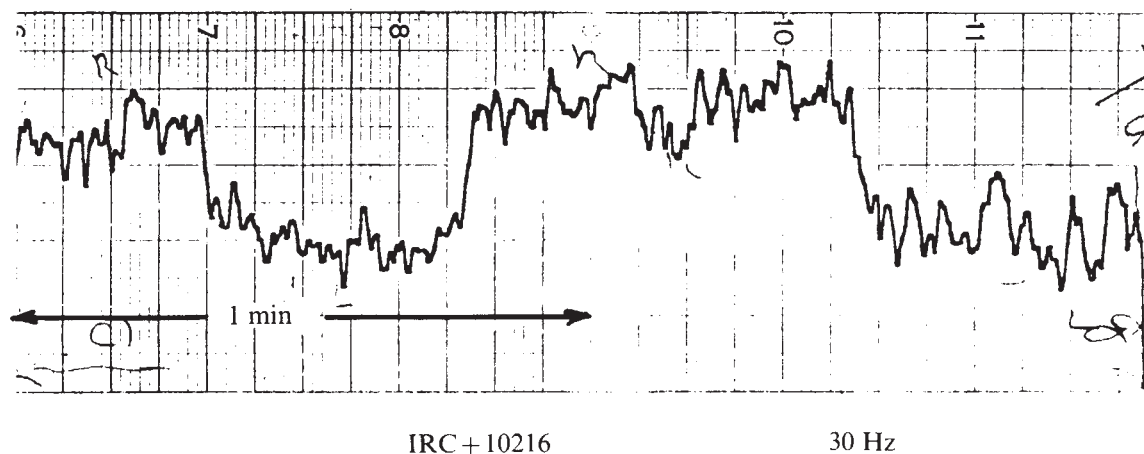
The Bent Cassegrain mode was used with the infrared photometer mounted in the aircraft cabin. Modulation and background calculations were achieved with an oscillating secondary mirror operated at 30 and 100 Hz. The half-power beamwidth was 0.9' and the separation between modulated beams was 2'.

Owing to mechanical difficulties with the aircraft, only one flight was made on March 9, 1976. Mars was observed for calibration

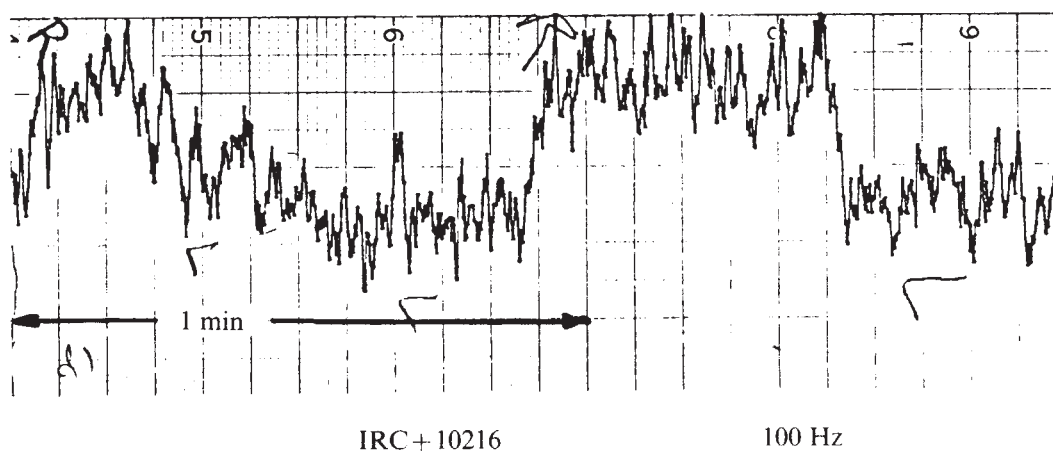
Fig. 1 *a.* Spectral response of Ge:Ga detector;



b. signal from IRC+10216 at 30 Hz;



c. signal from IRC+10216 at 100 Hz.



purposes for 5 min and IRC + 10216 for 30 min. Figure 1*b* and *c* shows the signals obtained from IRC + 10216 at modulation frequencies of 30 and 100 Hz; the two levels are produced by pointing the telescope so as to place the source in alternate beams. The apparent signal-to-noise ratio for both measurements was 6:1 but because of the wider recording bandwidth, the 100-Hz data are

in fact less noisy when normalised to unit bandwidth. The flux level for IRC + 10216 was obtained by assuming a brightness temperature of 200 K for Mars, whose semi-diameter at the time of observation was 39.9". Our 100- μm flux measurements of IRC + 10216 as shown in Table 1 agree with the measurements of Campbell *et al.*⁴. A unique aspect of this measurement is the use of

Table 1 100 μm flux measurement of IRC + 10216

Detector	Wavelength, λ_p (μm)	Bandwidth, $\Delta\lambda$ (μm)	Beam size ($''$)	Beam separation ($''$)	Measured flux (Jy)
Bolometer	100	40	1.4	2	2.460
Univ. of Arizona Photoconductor (Ge:Ga) N.R.L.	100	34	0.9	2	2.100

a photoconductive detector for the first time in an airborne infrared experiment. The sensitivity of our detection system is comparable with conventional bolometers that have been used in all airborne for infrared observations.

We thank the staff of the Gerard P. Kuiper Airborne Observatory for support and assistance. We thank Dr Hoffmann for his valuable comments and for reviewing our flight data. Drs D. A. Harper and R. F. Lowenstein of Yerkes Observatory provided technical assistance during the flight and initiated this study. This research was supported by the Office of Naval Research and by a NASA Grant.

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2.4- μm mapping of the galactic central region

OBSERVATION of the diffuse galactic radiation at near infrared wavelengths where the interstellar extinction becomes comparatively small could be an effective way of investigating the galactic structure especially in the central region of the Galaxy. We report here observation of the diffuse galactic radiation at 2.4 μm in the central region of the Galaxy between $l = 350^\circ$ and 30° using a balloon-borne telescope with a resolution of 1° . The central bulge of the Galaxy, and the very flat and narrow disk components were clearly demonstrated. A strong interstellar extinction concentrated in the galactic plane in the central region of the Galaxy was observed.

Our first observation by a balloon-borne telescope with wide spectral response (1.5–2.5 μm) was subject to unexpected temporal and spatial irregularities of the OH airglow emission¹. For near infrared observation of the diffuse light at balloon altitudes we thus limited the wavelength range to between 2.35 and 2.45 μm ; a unique band gap of the OH emission bands.

The successive observations were made on June 16, 1975 and May 31, 1976 by a 20-cm (f/1.25) balloon-borne telescope with two $5 \times 5\text{-mm}^2$ PbS detectors cooled by liquid nitrogen, each with a 1° square field of view, separated by 3.2° in elevation. The filters, which were also cooled, had an effective wavelength $\lambda_e = 2.4 \mu\text{m}$ and a bandwidth $\Delta\lambda = 0.08 \mu\text{m}$.

A malfunction of the chopping motor in the flight of 1975

limited the intensity profile across the galactic plane at $l = 6^\circ$. During the flight on May 31, 1976, we scanned the extended area along the galactic plane between $l = 350^\circ$ and 30° within latitudes of $[b] \leq 10^\circ$ in such a way that the elevation angle was stepped up by 0.9° after each strip scan of 30° width in azimuth.

The direction of the telescope was assigned to celestial coordinates with the aid of a geomagnetic aspectometer for the azimuth sensor and an elevation meter with a potentiometer. Definite detections of several bright IRC stars facilitated a check on the accuracy of the position determination, estimated to be $\pm 0.3^\circ$ throughout the observation.

The absolute responsivities of the detectors were calibrated by observing α Sco ($m_k = -3.87$) (ref. 2) and also monitored by an artificial calibration light during the flight. The contribution of nearby infrared objects, a single or a group of IRC stars, was subtracted by referring to K -magnitudes in the infrared catalogues^{3–5}.

The two detectors yielded independent values for the brightness distribution, and the consistency between these was satisfactory. The brightness values were synthesised into a map (Fig. 1). The data beyond $l = 27^\circ$ may be less reliable because of a fault arising in the transmitter system, so the map has been limited within $l = 27^\circ$. Our map tends to be smoothly linked to that presented by Hayakawa *et al.*⁵.

The marked concentration of the surface brightness toward the galactic centre clearly implies that the central bulge or the spheroidal stellar distribution of our Galaxy has been detected. This constitutes the first demonstration of the deep inner structure of our Galaxy. The apparent shape of the central bulge must be subject to the interstellar extinction which should be highly concentrated in a narrow zone along the galactic plane. This is clearly indicated by the fact that the highest contours close to the galactic centre are prolonged not along the galactic equator, but perpendicular to it. The 2.4- μm brightness at the galactic centre is $1.6 \pm 0.03 \times 10^{-9} \text{ W cm}^{-2} \text{ sr}^{-1}$.

Figure 2 shows the 2.4- μm brightness distribution along the galactic equator, the 2.6-mm CO line intensity⁶ and the 100- μm brightness⁷. The near infrared profile seems to consist of two components, the central spheroidal structure and the flat disk component. Flatness beyond $l = 10^\circ$ is remarkable, extending to the sudden decline near $l = 30^\circ$, which was found by Hayakawa *et al.*⁵. This would imply a strong concentration of infrared sources in the 5-kpc ring as discovered from the CO observations⁸. Thinness of the disk component (4° in FWHM) is also prominent.

The concentration of the spheroidal component toward the galactic centre is much less steep compared with that observed in M31 in infrared^{9,10}, and in visual brightness distributions¹¹. If the intrinsic brightness distribution of the central region of our Galaxy is the same as that of M31, the difference could be attributed to obscuration by a dense dust layer lying in the central region of the Galaxy. This dust layer extends mainly within $b = \pm 1^\circ$, with a slight concentration towards the galactic centre. The total amount of the extinction in direction to the galactic centre is about 2.4 mag at 2.4 μm , corresponding to $A = 30$ mag, if we assume the general wavelength dependence of the interstellar extinction. It is almost compatible to the value obtained by Becklin and

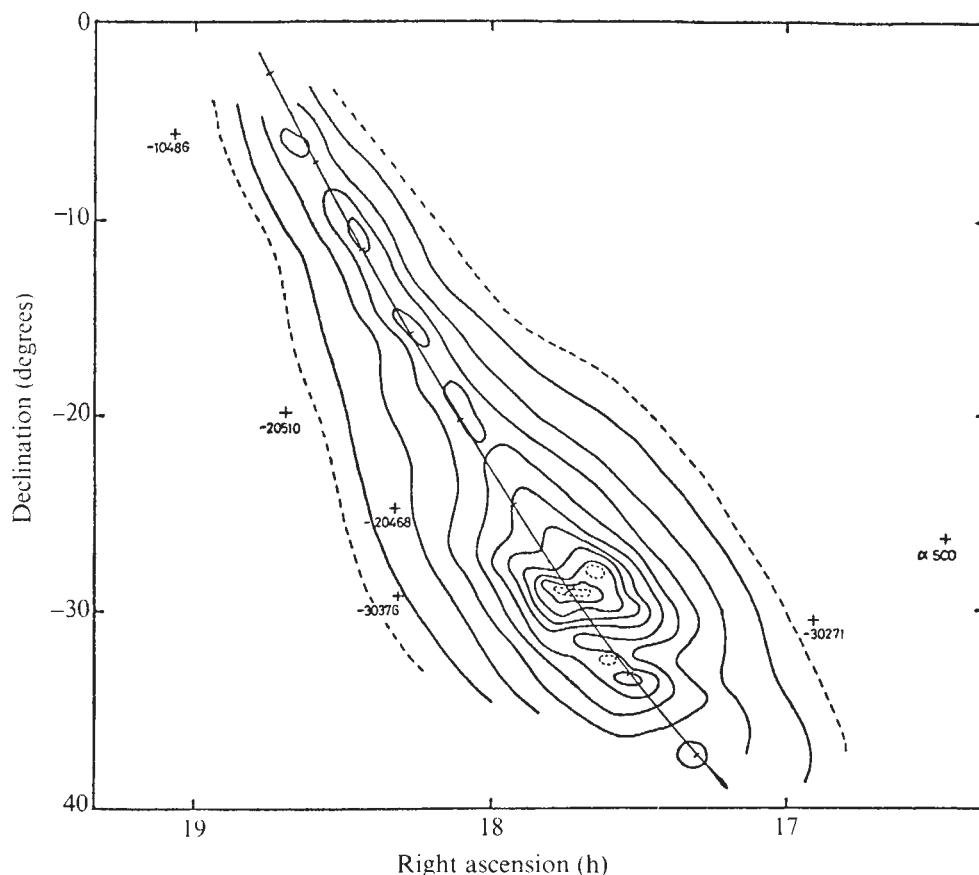
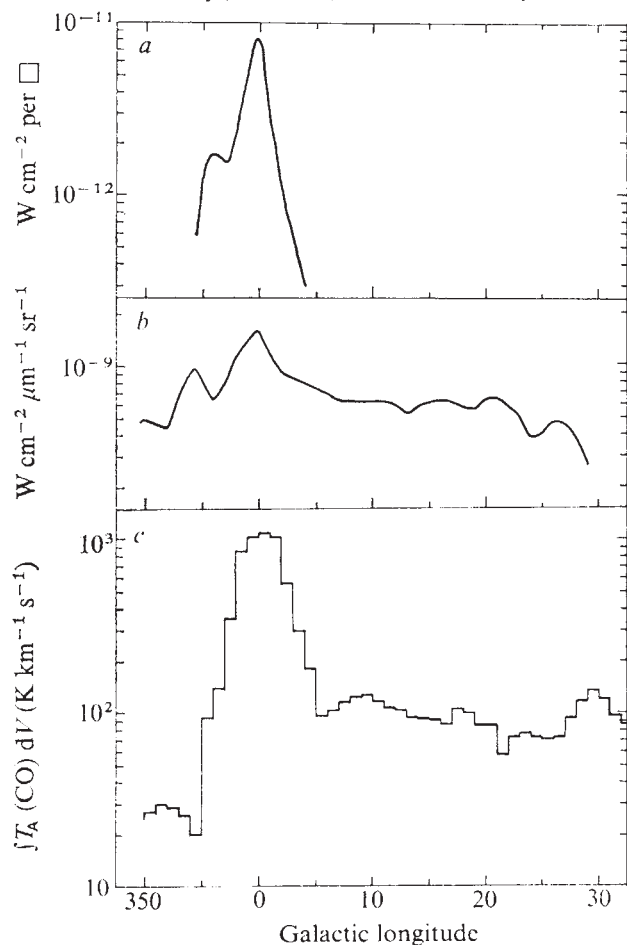


Fig. 1 2.4- μ m map of the galactic diffuse light. Unit of the contour is $1.6 \times 10^{-10} \text{ W cm}^{-2} \mu\text{m}^{-1} \text{ sr}^{-1}$, dashed contours indicate half interval. Crosses indicate the positions of IRC stars used for the position calibration.

Fig. 2 b, Longitudinal dependence of the 2.4- μ m intensity along the galactic equator. Those of *a*, the 100- μ m intensity, and *c*, the CO intensity ($J = 1 \rightarrow 0$) are shown for comparison.



Neugebauer¹². About one third of the extinction is attributed to the dust layer in central region of the Galaxy, that is, within $l = \pm 2^\circ$. This dust layer corresponds well to that detected as the extended far infrared source by Hoffmann *et al.*¹³ and Soifer and Houck⁷, and may be related to the CO molecular clouds concentrated in the galactic centre.

It is noticeable that there is an asymmetry between positive and negative sides of the galactic longitude. A dip near $l = 357^\circ$ may correspond to the subsidiary peak in the 100- μ m profile. A peak near $l = 354^\circ$ is also interesting. Open clusters NGC 6383 and 6405 (M 6) locate at the same position but seem to be insensitive to the 2.4- μ m brightness. Similar features have been found in the balloon observation made by the infrared group of Nagoya University (T. Matsumoto, personal communication).

Whether these features are caused by variation in local interstellar obscuration or by some hidden objects should be clarified by further investigation.

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Infrared profile of central region of our Galaxy at $2.47\text{ }\mu\text{m}$

SEVERAL investigators have described the features around the galactic centre in some infrared wavelength regions¹⁻³. Their results demonstrate the conspicuous peak and several humps around it. These observations, however, are confined to a rather small region mainly because of the terrestrial background radiation. So the overall feature of the central bulge of our Galaxy could not be derived. Studies of the central bulge have been carried out only with the aid of the distribution of RR Lyrac variables

through the 'galactic window'⁴, but the distribution does not necessarily represent that of general stars which are responsible for the galactic structure. On the contrary, near infrared observation reveals the structure of the central bulge more directly, because the main constituents of the bulge are probably late-type stars which radiate their energy effectively in the infrared region, as inferred from external galaxies. Much weaker interstellar extinction in the infrared region also enables us to observe the whole central bulge through thick interstellar dust clouds. The surface brightness at $2.4\text{ }\mu\text{m}$ in the range of $75^\circ > l > 23^\circ$, $|b| < 20^\circ$ was attained by our previous balloon experiment^{5,6}. We have now attempted further similar observations aiming mainly to derive the surface brightness of the central bulge of our Galaxy.

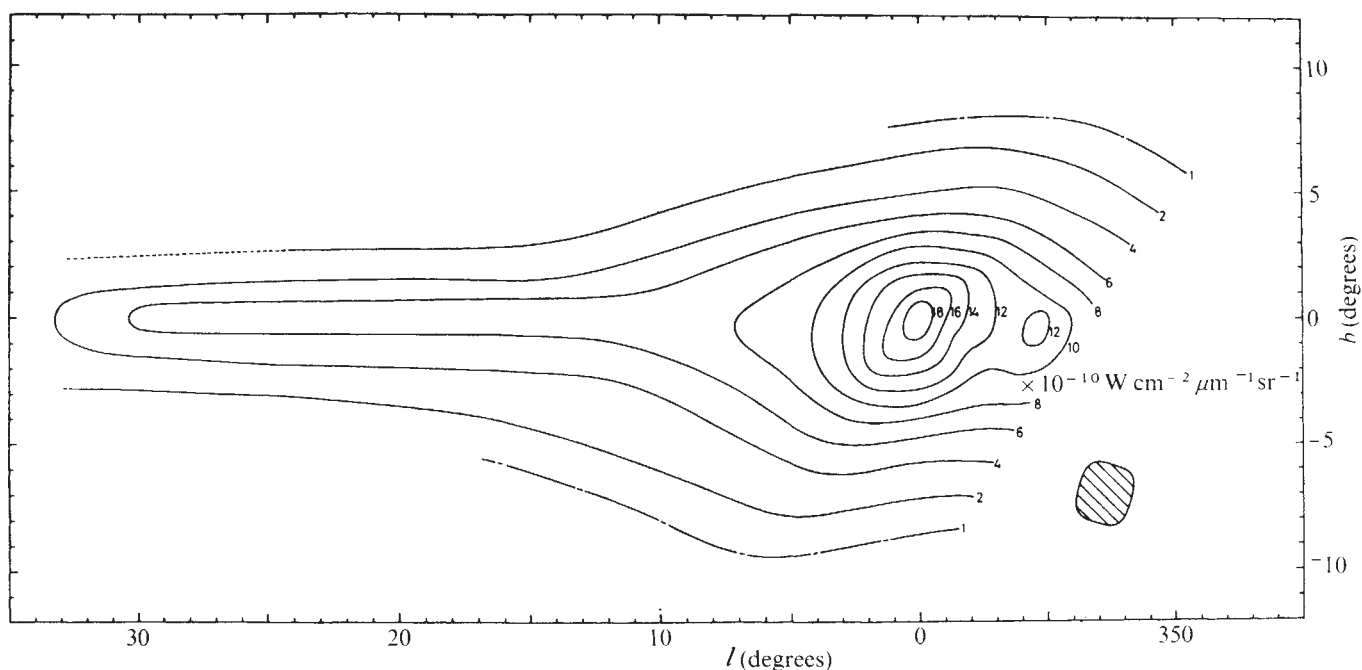


Fig. 1 Isophoto of the observed surface brightness of our Galaxy. The shaded area (bottom right) indicates the field of view used (HMBW).

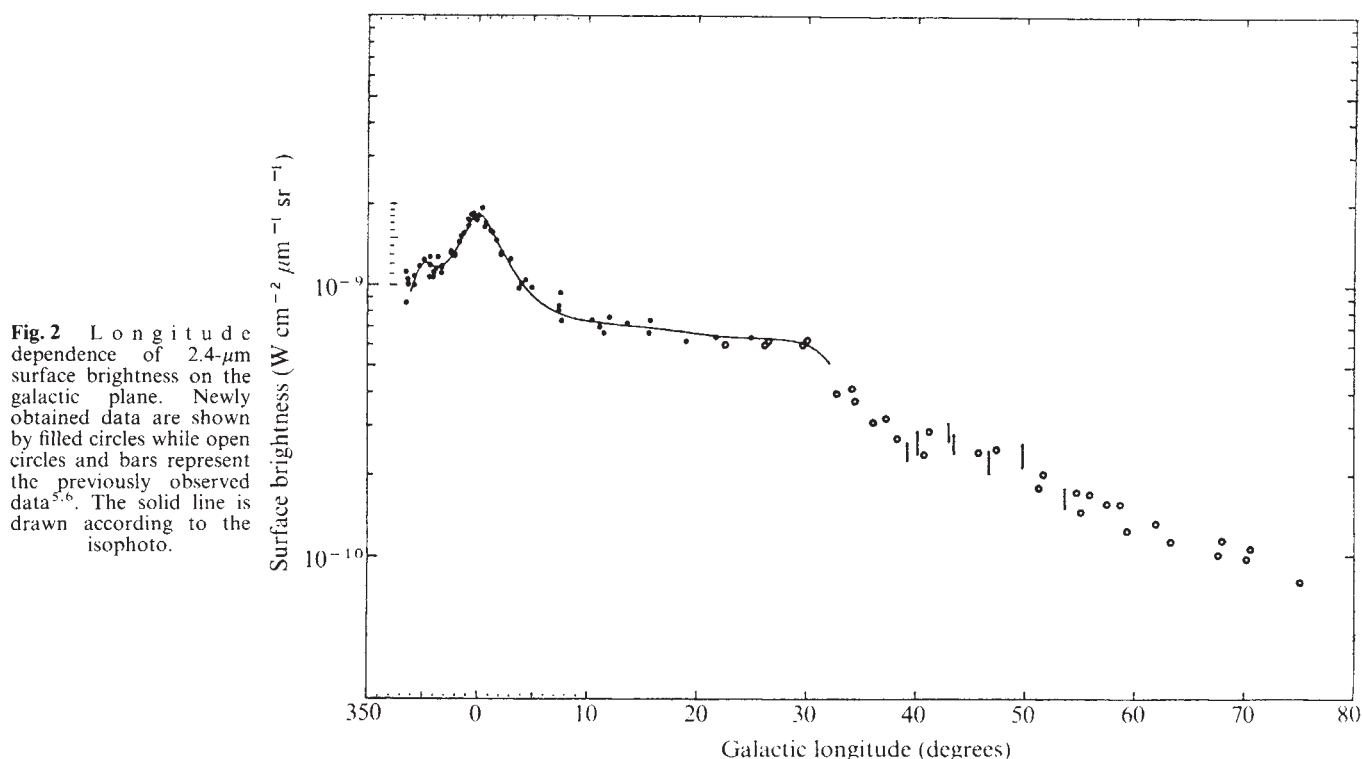


Fig. 2 Longitude dependence of $2.4\text{-}\mu\text{m}$ surface brightness on the galactic plane. Newly obtained data are shown by filled circles while open circles and bars represent the previously observed data^{5,6}. The solid line is drawn according to the isophoto.

The balloon-borne infrared telescope was similar to that used for the previous flight. It consisted of a silicon combination lens system ($D = 10$ cm, $F = 1.0$) in front of which an interference filter ($\lambda = 2.4$ μm , $\Delta\lambda = 0.1$ μm) was attached, and an array of four lead sulphide detectors installed at the focal plane, each of which generated the field of view of $2^\circ \times 2^\circ$. The whole system was cooled by liquid nitrogen to reduce the thermal background radiation and improve the detector sensitivity.

The instrument on board a balloon was launched at 1850 JST on May 30, 1976 from Sanriku Balloon Center of the Institute of Space and Aeronautical Science, University of Tokyo.

The whole system functioned as expected during the flight and a celestial region of $35^\circ \geq l \geq -7^\circ$, $|b| < 15^\circ$ was covered by 72 scans. These scan paths were derived from the output of the magnetometer and confirmed by the transit of α Sco across the field of view. The observation of α Sco also provided an absolute calibration. The flux 1.38×10^{-12} $\text{W cm}^{-2} \mu\text{m}^{-1}$ of α Sco at 2.4 μm was provided by the balloon observation on the previous night⁷. The responsiveness of the detector systems was checked by lighting a calibration source.

The infrared surface brightness thus obtained is shown in the form of an isophoto in Fig. 1. The scan paths are rather concentrated into the range of $-10^\circ < l < 10^\circ$, so that the contour lines in that region are more accurate than in the rest. The contours beyond $l = 30^\circ$ are drawn with reference to previous data. The longitude dependence at $b = 0^\circ$ and latitude dependence at $l = 0^\circ$ are shown in Figs 2 and 3, respectively. The present result agrees well with our previous one between $l = 20^\circ$ and 25° .

At a glance the isophoto shows that the galactic radiation at 2.4 μm consists of two components: disk component and bulge component. The surface brightness and width of the plane are nearly constant in the region $10^\circ < l < 30^\circ$ with the value of 6.5×10^{-10} $\text{W cm}^{-2} \mu\text{m}^{-1} \text{sr}^{-1}$ and 4.5° (f.w.h.m.) respectively.

The bulge component extends in the region $l < 5^\circ$ which corresponds to 1 kpc from the centre. If we exclude the hump at $l = 355.5^\circ$, $b = -0.5^\circ$ and absorbing lane from centre to

Fig. 3 Latitude dependence of 2.4 - μm surface brightness at $l = 0^\circ$.

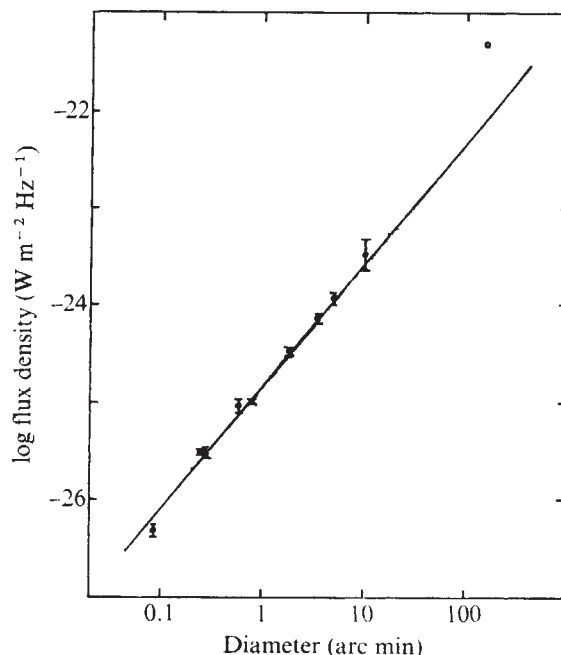
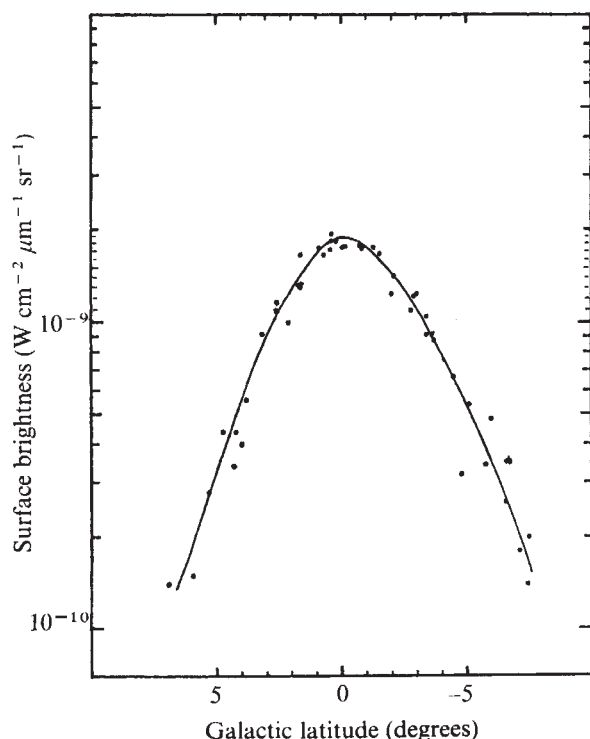


Fig. 4 The relation between flux density of the galactic centre and aperture diameter employed. Filled circles and straight line are taken from ref. 1. An open circle at upper right represents our value.

$l = 357.5^\circ$, $b = -2.5^\circ$, the bulge component is almost symmetrical with respect to the galactic centre.

The flux of the galactic centre of $(1.9 \pm 0.1) \times 10^{-9}$ $\text{W cm}^{-2} \mu\text{m}^{-1} \text{sr}^{-1}$ is compared with ground-based observations at 2.2 μm in Fig. 4. Our value is rather larger than that extrapolated from the small aperture, since our value contains the disk component and the large aperture results in smaller interstellar extinction because of strong concentration of the absorbing matter on the galactic plane.

The contour lines within 2 arc deg from the centre in Fig. 1 are elongated in the direction perpendicular to the galactic equator, and in the outer region the lines are elongated parallel to the galactic plane. The former can be attributed to the interstellar absorption, and the latter probably reflects the property of the central bulge.

The hump at $l = 355.5^\circ$, $b = -0.5^\circ$ in Fig. 1 is considered to be an extended source, since there are no corresponding bright stars at 2.2 μm around the position of the hump⁸. The open cluster NGC 6383, which is located at the almost same position, cannot contribute to the 2.4 - μm surface brightness. A radio source which may correspond to the hump is not also found in this region. It is uncertain at the present time, however, whether the hump is related to the structure of the central bulge.

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Disappearance of puddle water during the night with growth of ice needles and its reappearance during the day

A PUDDLE appears in a dip in the ground after a fall of rain or snow. It becomes covered with an ice sheet during cold nights in the winter in the rural area of the northern part of the Kanto district in Japan. The ice sheet has a characteristic shape. It is not flat but dished and usually an air space is found under thin ice sheets without puddle water. Furthermore, an abundance of ice needles grow on the soil surrounding the puddle. These phenomena in puddles in winter seem to be general in the Kanto district. This report describes observations made on some puddles located at the side of the road in the Kanto district during February 1976 to clarify the process of formation of the dished-out ice sheet, the air space under the ice sheet, and the disappearance and appearance of puddle water.

Photographs of the various states of puddle water obtained on February 7–8 are shown in Fig. 1a–f. In the daytime the puddles were not covered with an ice sheet (Fig. 1a) though they were covered with ice sheets early in the morning before the increase in air temperature (Fig. 1b).

Stripe patterns were found in the ice sheet. Under the ice sheet the puddle water was replaced by air (Fig. 1c). A cross section of the ice sheet showed the difference of ice thickness with position (Fig. 1d). The stripes observed on the ice sheet are due to the difference in ice thickness.

Abundant ice needles 3–5 cm long were observed on the soil surrounding the puddle (Fig. 1e). Furthermore, melting of the ice needles and an increase in puddle water under

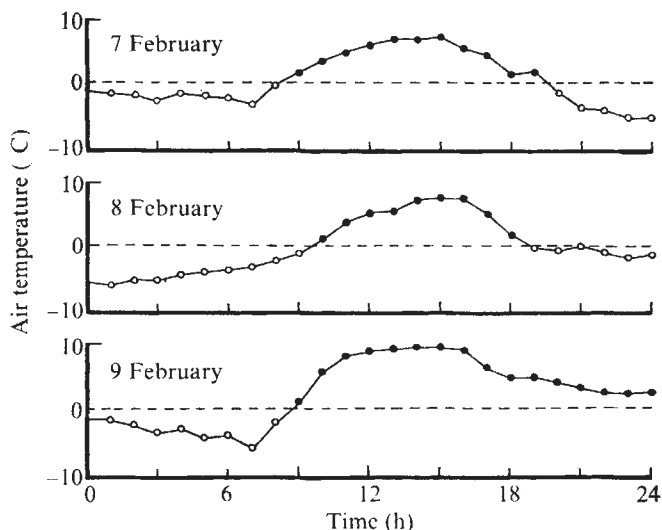


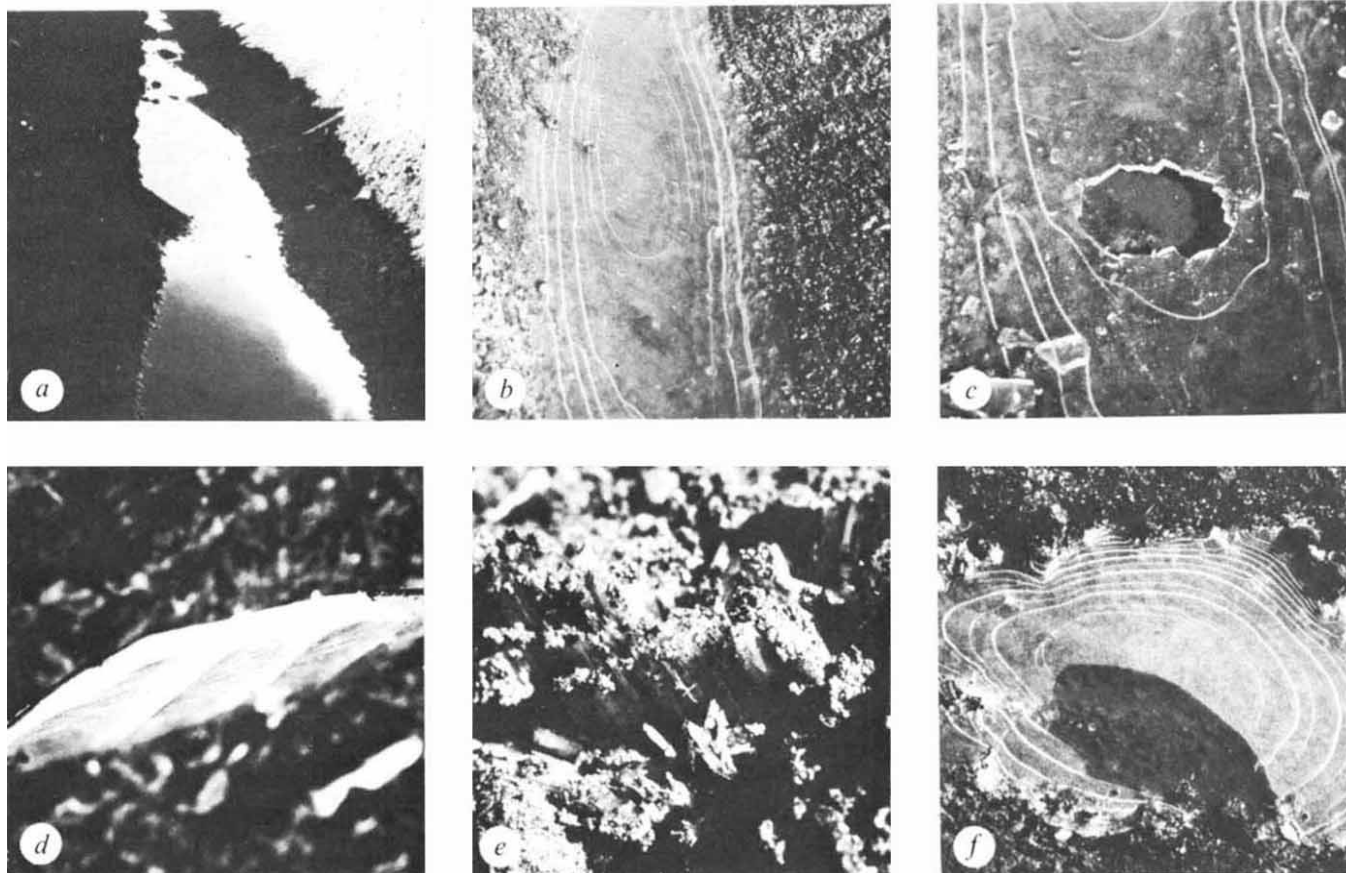
Fig. 2 Air temperature variation about 300 m from observation area.

the ice sheet were observed in the morning with the increase in solar radiation.

The air temperature data obtained at the Tateno Aerological Observatory about 300 m from the observed puddles on February 7–9, 1976 showed the cycle of air temperature above 0 °C from 0900 to 1900 and below 0 °C from 2000 to 0800 (Fig. 2).

These observations suggest that the following events occur. The growth of ice needles around the puddle is due to the supply of water from the puddle followed by the

Fig. 1 Photographs of puddle water at various times of day.



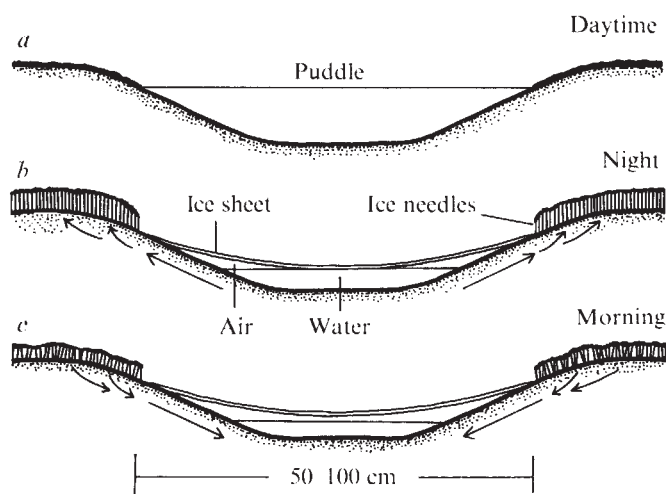


Fig. 3 Air space formation beneath puddle ice sheet.

decrease of puddle water. The decrease of puddle water results in the gradual lowering of the level of formation of the ice sheet which occurs on the surface of puddle water. This is the reason for the formation of the dished ice sheet. And then, with the continuation of the growth of ice needles, the puddle water separates from the ice sheet to form an air space (Fig. 3). The irregularity of the growth of ice needles controlled by the delicate balance of meteorological conditions of air and soil¹⁻⁷ may be the cause of the striped patterns of the ice sheet. With the increase of solar radiation in the morning, the ice needles and ice sheet melt and water returns to the puddle.

After the melting of the ice needles and ice sheet, the puddle water regains its previous daytime shape.

The puddles formed at the side of road after a fall of rain or snow in winter repeat this process with the evaporation of puddle water. The area of water evaporation from the surface of puddle water is extended by the penetration of the water into the soil surrounding the puddle. This results in more rapid disappearance of puddle water from the dip in the ground. The periods of existence of the puddles which we observed were about 10 days.

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Measurement of forces of molecular attraction of crossed fibres as a function of width of air gap

DERJAGUIN and Abrikosova¹ were the first to measure molecular forces as a function of the distance between macro-objects, using negative feedback. The results coincided, within the limits of experimental error, with the conclusions of the theory of retarded molecular forces (which was subsequently developed

by Lifshits^{2,3}). In the earlier research work¹, as in a number of subsequent experiments, the width of an air gap was measured by optical methods (using the interference of light). The research work was therefore limited to transparent bodies. Moreover, test bodies had to possess a sufficiently large radius of curvature. The latter condition meant that the quality of the surfaces had to meet stringent requirements; it was also difficult to ensure the absence of dust particles in the gap between interacting bodies with large radius of curvature. As a result, only in two cases^{4,5}, owing to the use of molecular-smooth surfaces of mica, did researchers succeed in measuring, not only the retarded, but also non-retarded molecular forces.

In the present work, we have used fibres of small radius (about 150-500 μm) crossed at right angles. With a view to presetting the gap, instead of measuring it directly (including that between opaque bodies), we preset the displacement of one of those bodies relative to the position of the contact with another fixed body. (The contact we assumed to be that position of fibres, which corresponds to the contact interaction force $F = 0$). To this end, a movable fibre was attached to the frame of a photoelectric galvanometer and another fibre was fixed.

The light ray reflected by the galvanometer mirror impinged on two photoresistances. When the instrument was operating normally as a galvanometer, the equilibrium position of the frame corresponded to the equality of illumination of those photoresistances. In the case of a deviation from the equilibrium, current I_1 passed through the diagonal of the bridge circuit, and a part of current I_1 , current I_2 through the galvanometer frame. Owing to the negative feedback, the current I_2 reset the frame in its equilibrium state. In addition to the two photoresistances, the bridge circuit comprised two constant resistances S_1 and S_2 . When the frame was in equilibrium, $I_1 = 0$.

The instrument was remodelled by replacing the constant resistances S_1 and S_2 by the variable ones, to measure the forces of interaction of fibres. To this end, multistage magazines of resistances were used. By varying S_1 and S_2 , one could vary the illumination ratio of the photo-resistances, corresponding to $I_1 = 0$, and, hence, the equilibrium position of the frame. The readings S_1 and S_2 were converted into the values of displacements of the movable thread by the preliminary graduation. The currents I_1 and I_2 were measured by microammeters.

Each equilibrium position of the movable thread was preset by the values of S_1 and S_2 ; in the absence of a force applied to the thread this corresponded to $I_1 = 0$.

When the interaction forces appeared, this equilibrium state was violated. An electric current $I_1 \neq 0$ flowed through the diagonal of the bridge circuit. To compensate for it, an independent controllable power source was used, which was additionally cut in the circuit of the galvanometer frame. The value of the current, I_2 , was proportional to the forces applied to the thread. The instrument had been previously graduated, to convert the values I_2 into the force value.

Thus, the space position of the moveable fibre, which was read off the contact point of fibres, was determined by the values S_1 and S_2 at $I_1 = 0$. In this case, the force of interaction of the fibres was determined according to the current I_2 . Consequently, the circuit applied enabled us, using only one device, to measure both the spacings between the fibres and their interaction forces.

As a result, we have succeeded in presetting the shift of fibres at an accuracy of up to 10 $\text{\AA}$. The position of the contact was defined at an accuracy of up to 50 $\text{\AA}$. The sensitivity of the instrument to the interaction force was about 10^{-4} to 10^{-5} dyne.

The forces of interaction of quartz, glass, platinum and gold fibres were measured as a function of the shortest distance h between them, within the range of 100-1,000 $\text{\AA}$. In the case of dielectrics, a β -radiation source was used to remove electrostatic charges.

The theory of molecular forces^{2,3} gives the following relationships for the forces of attraction of cylinders crossed at

right angles and having radii R_1 and R_2

$$F_1 = 2\pi B \sqrt{(R_1 R_2)/3h^3} \quad (1)$$

$$F_2 = A \sqrt{(R_1 R_2)/6h^2} \quad (2)$$

Equation (1) corresponds to the case of retarded forces, and equation (2) to that of non-retarded forces. Here A and B are the constants of molecular forces.

To compare the experimental results obtained with fibres of different diameters, it will be more convenient to use the interaction energy u per unit area of the plane-parallel surfaces separated by the same gap h . In accordance with ref. 6,

$$u = \frac{F}{2\pi \sqrt{(R_1 R_2)}}; \quad u_1 = \frac{B}{3h^3}; \quad u_2 = \frac{A}{12\pi h^2} \quad (3)$$

Unlike F , the values u do not depend on the radius of the cylinders that are used to measure the force.

Figure 1 shows the relationships $u(h)$ obtained for quartz and platinum fibres. Each point on the curve represents a result of averaging several experimental values of u with a definite value h . Solid straight lines were plotted by the least squares method (over the entire field of points, but not according to the averaged results), taking into account the square or the cube dependence of u on $1/h$.

Using the same plurality of experimental points as the basis, we were able to determine the exponent n , without having recourse to the relationships (1) and (2) (assuming that $u \sim 1/h^n$). For quartz, the values n were found to be equal to $n_1 = 3.01$ and $n_2 = 2.0$; for platinum, to $n_1 = 3.03-3.23$ and $n_2 = 1.93-2.01$. This agrees well with theory. At $h \lesssim 300$ Å, platinum deviates from the dependence $u \sim h^{-2}$; this is probably attributable to the roughness of the surface of fibres.

From the results represented in Fig. 1, it was easy to determine the values of constants A and B , as well as to evaluate the gap width h_0 , at which the non-retarded molecular forces go over into the retarded ones.

Table 1 gives the values of A , B and h_0 for quartz and platinum. The values of A and B obtained agree satisfactorily with the calculated ones⁷. In the case of quartz, those values are close to the measured results obtained by other methods:

Table 1 Results of measurements of A and B

Material	$A \times 10^{12}$ (erg)		$B \times 10^{19}$ (erg cm)		h_0 (Å)
	Expt	Theory	Expt	Theory	
Fused quartz	0.45	0.8	1.02	0.6	270
Platinum	2.0	—	10.5	13.0	650

$A = 6.5 \times 10^{-13}$ erg, $B = (0.66-1.15) \times 10^{-19}$ erg cm (ref. 6). We were the first to obtain the experimental values B for platinum. The values A for platinum were calculated earlier, using the results of experiments made with crossed fibres in electrolyte solutions⁸: $A = (0.8-1.6) \times 10^{-12}$ erg.

Hence, the method thus worked out allowed the molecular forces to be measured as a function of the spacings between the fibres in air at a distance of about 100–1,000 Å between various materials, including these opaque ones. The experimental results agree well with the theory.

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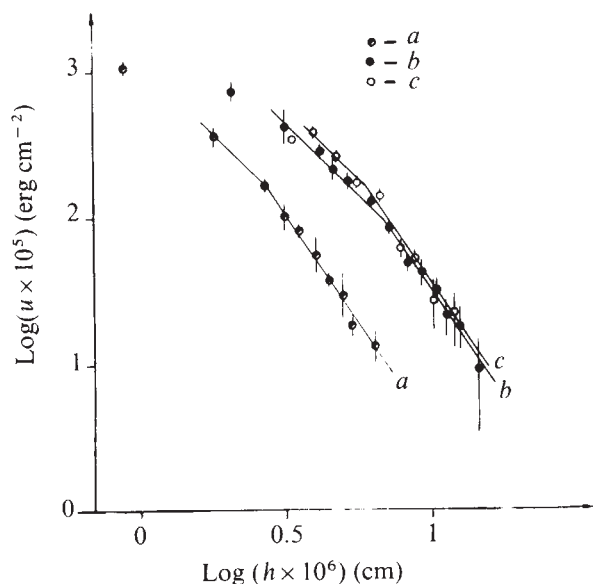
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Fig. 1. Dependence of the specific energy of the molecular attraction of plane-parallel surfaces, u , on distance h . *a*, Quartz, $2R_1 = 0.8$ mm, $2R_2 = 1.15$ mm. *b*, Platinum, $2R_1 = 1$ mm, $2R_2 = 0.3$ mm. *c*, Platinum, $2R_1 = 2R_2 = 0.3$ mm.



Cross relaxation and spin diffusion in the proton NMR of hydrated collagen

PROTON NMR of water is being used extensively to probe the molecular dynamics of water molecules in biological systems such as protein solutions, hydrated macromolecules, cells and tissue. The nuclear magnetic relaxation rates R_1 (spin-lattice) and R_2 (spin-spin) can be analysed in terms of the rotational motions of the water molecules¹⁻³. A crucial assumption in this analysis is that the proton relaxation of the water proceeds independently of that of the macromolecules. Kimmich and Noack⁴⁻⁶ claim that this assumption may be incorrect for proton spin-lattice relaxation because of spin diffusion, but clear evidence of spin diffusion in hydrated biological samples has not been reported. We show here that the proton spin-lattice relaxation behaviour in hydrated collagen is dominated by cross relaxation between the water protons and the macromolecular protons as a result of spin diffusion; the macromolecular spin-lattice relaxation contributes significantly to the water proton R_1 .

The proton spin-lattice relaxation of ribbons of reconstituted collagen⁷, hydrated with H_2O or D_2O , was studied by pulsed NMR. The spin-lattice relaxation was determined from a comparison of the free induction decay (FID) signal after a $(180^\circ - t - 90^\circ)$ pulse pair with that after a single 90° pulse. The pulse pair yields the partially relaxed magnetisation $M_z(t)$ whereas the single 90° pulse yields the equilibrium magnetisation M_0 . The spin-lattice relaxation rate was determined from a logarithmic plot of the reduced magnetisation $m(t) = -(M_z(t) - M_0)/2M_0$ against t .

The FID of hydrated collagen consists of two components (see Fig. 1). Protons in the rigid collagen matrix constitute a

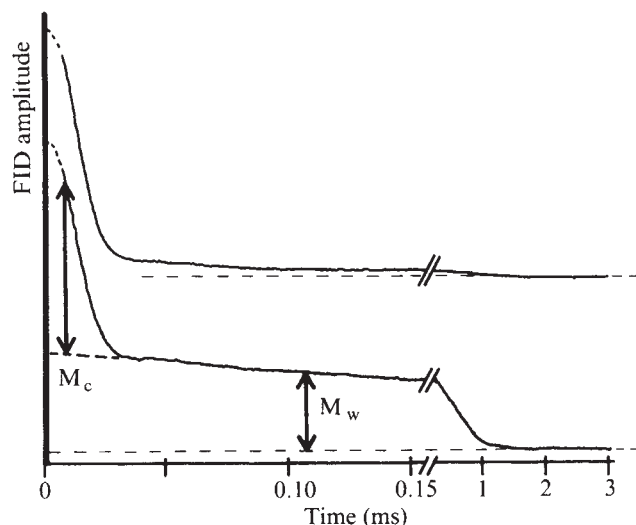


Fig. 1 Proton FID signals in reconstituted collagen, hydrated with 28 g water per 100 g collagen. Lower curve: hydrated with H_2O . Upper curve: hydrated with 95% D_2O . The collagen signal intensity $M_c(t)$ was measured immediately after receiver recovery from the 90° pulse, 10 μs after the beginning of the pulse. The water signal intensity $M_w(t)$ was measured 60, 110 and/or 400 μs after the pulse; the spin-lattice relaxation decay at these times is the same. The collagen fibres were oriented perpendicular to the magnetic field. Measurements were performed at room temperature with a Bruker pulse spectrometer operating at 30 MHz; a Varian V-2708 magnet was used, equipped with a Varian V-3508 flux stabiliser. The 90° pulse length was 1.5 μs . The FID signals were averaged with a combination of a Biomation 802 signal digitiser and a Nicolet 7002 signal averager. Typically 64 or 128 repeated measurements were averaged together.

fast relaxing part ($R_2^* = 60,000 \text{ s}^{-1}$), and the water protons relax much more slowly ($R_2^* \lesssim 5,000 \text{ s}^{-1}$). This identification of the two signal components is supported by the observations that the intensity of the water fraction changes in direct proportion to the amount of hydration water and to the isotopic dilution with D_2O , and that the relative intensities of the two fractions correspond to the calculated ratio of collagen protons to water protons (three to seven, respectively, for the sample shown in Fig. 1). No static dipolar splitting of the water signal was apparent, in agreement with other studies of natural collagen under similar conditions^{8,9}.

The two components of the FID can easily be separated (see Fig. 1) so that we could study the spin-lattice relaxation of the water and of the macromolecular protons separately (Fig. 2). Measurements at different water contents all showed the following characteristics (see Fig. 2): (1) The relaxation rate R_1 of both water and collagen protons is the same if the initial 10 ms of the relaxation decays is ignored. (2) The beginning of the water relaxation decay is clearly concave (curve c) and that of collagen is convex (curve b). Both relaxation curves are described by a sum of two exponentials. (3) When collagen is hydrated with D_2O , then the relaxation rate of the collagen protons is single-exponential and proceeds at a rate which is much slower than in the presence of H_2O (curve a).

Proton NMR relaxation is brought about by magnetic dipolar interactions. Substitution of D_2O for H_2O changes the nuclear dipolar interactions between collagen protons and water nuclear spins. Assuming that the dynamics of hydration are similar for H_2O and D_2O , then the dipolar interaction between a collagen proton and a water proton is of the order of $(\gamma_H/\gamma_D)^2 (I_H/I_D) \times (I_H+1)/(I_D+1) = 16$ times more effective at inducing nuclear spin transitions than the interaction between a collagen proton and a water deuteron¹⁰. The large change in R_1 of the collagen protons on substitution of D_2O for H_2O clearly indicates that the macromolecular protons 'feel' the presence of water protons. Consequently, dipolar coupling

between water and macromolecular protons must be an important factor in the proton relaxation mechanism in hydrated collagen.

The non-exponentiality of the water relaxation might be ascribed to the presence of two slowly exchanging water fractions. This seems unwarranted, however, as it does not explain the convex curvature in the collagen relaxation, or account for the similar relaxation rates of the two components after the initial 10 ms of decay. We can explain all of our observations by realising that the dipolar coupling between water and collagen protons provides a way for cross relaxation between the water and the collagen protons by the spin diffusion mechanism^{10,11}.

We will assume that the protons in the water phase as well as in the collagen phase have, at any time, a uniform spin temperature, that is, the longitudinal magnetisation $M_z(t)$ in each phase is uniform. Within the collagen phase spin diffusion among the collagen protons will rapidly establish a common spin temperature, whereas in the water phase this is accomplished by rapid chemical exchange. The water phase may also include exchangeable collagen protons. Let p_w and p_c be the fraction of protons in the water phase and in the collagen phase respectively, with the proton relaxation rates in the absence of cross relaxation denoted by R_{1w} and R_{1c} . It should be noted that R_{1w} comprises the contributions to the water proton relaxation rate which are normally included in theoretical treatments, for example, effects from bound and free fractions of water¹⁻³. Whenever a proton of the water phase resides near a collagen proton, the two protons are coupled by the magnetic dipolar interaction and a mutual spin flip can occur¹⁰. Such a spin flip exchanges spin energy between the two proton phases,

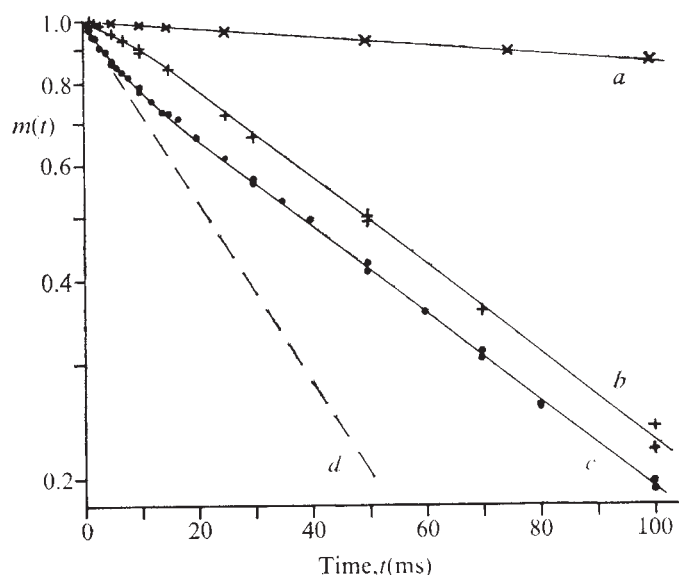


Fig. 2 Proton spin-lattice relaxation decays for the reduced magnetisation $m(t)$ in hydrated reconstituted collagen for the same samples as in Fig. 1. Measurements of $M_z(t)$ and M_0 were alternated and the FID signals were averaged into two halves of the memory of the signal averaging system. This alternation ensures very accurate R_1 measurements since slow changes in time of signal gain and/or of field drift affect $M_z(t)$ and M_0 in the same way and are compensated. Curve a, $m_c(t)$ for the collagen proton signal, hydrated with 95% D_2O ($\times$). Curve b, $m_c(t)$ for the collagen proton signal, hydrated with H_2O ($+$). Curve c, $m_w(t)$ for the water proton signal of H_2O ($\bullet$). A least squares fit of equation (2) to $m_w(t)$ yields the estimates, $R_1 = 15 \pm 1 \text{ s}^{-1}$; $c_w^- = 0.89 \pm 0.01$; $R_1^+ = 160 \pm 15 \text{ s}^{-1}$; $c_w^+ = 0.11 \pm 0.01$. Curve a gives $R_{1c} = 3 \pm 0.5 \text{ s}^{-1}$. With $p_w = 0.3$, equation (3) gives $R_{1w} = 31 \pm 4 \text{ s}^{-1}$; $k_c = 40 \pm 10 \text{ s}^{-1}$; $k_w = 100 \pm 25 \text{ s}^{-1}$; $c_c^- = 1.08 \pm 0.01$; $c_c^+ = -0.08 \pm 0.01$. Solid lines represent the theoretical decays obtained with these values of the parameters. Curve d represents the water relaxation decay with a relaxation rate $R_{1w} = 31 \text{ s}^{-1}$ as it would occur in the absence of cross relaxation.

leading to cross relaxation between the two phases. Spin diffusion may occur through protons on water molecules that are temporarily bound to the collagen, or through rapidly exchanging collagen protons. Spin diffusion will be effective in particular, if such bound nuclei exchange at a rate which is slow compared to ω_n , the NMR measuring frequency^{10,11}.

We can introduce a cross relaxation rate k_c , the rate at which magnetisation diffuses from the collagen to the water; k_w is the reverse rate. We can then write a set of modified Bloch equations, similar to those well-known for the case of chemical exchange:

$$dm_w(t)/dt = -R_{1w}m_w(t) - k_w m_w(t) + k_c m_c(t) \quad (1a)$$

$$dm_c(t)/dt = -R_{1c}m_c(t) - k_c m_c(t) + k_w m_w(t) \quad (1b)$$

Unlike the case of chemical exchange, (1) applies only to the z-component of the magnetisation; spin diffusion does not lead to cross relaxation in the transverse magnetisation.

The solution of the set of coupled differential equations (1) predicts that the relaxation in either phase is described by two apparent relaxation rates R_1^+ and R_1^- which are the same for both phases. The complete solution¹² is (the subscript i refers to either of the phases)

$$m_i(t) = c_i^+ \exp(-R_1^+ t) + c_i^- \exp(-R_1^- t) \quad (2)$$

where

$$R_1^\pm = \frac{1}{2}(R_{1c} + R_{1w} + k_c + k_w) \pm \frac{1}{2} \sqrt{[(R_{1c} - R_{1w} + k_c - k_w)^2 + 4k_c k_w]} \quad (3a)$$

$$c_i^\pm = \pm (R_1^\mp - R_{1i}) / (R_1^- - R_1^+) \quad (3b)$$

$$k_w = (p_c/p_w)k_c \quad (3c)$$

From (3b) follows

$$R_{1i} = c_i^+ R_1^+ + c_i^- R_1^- \quad (3d)$$

With these expressions all the characteristics of the spin-lattice relaxation can be explained quantitatively.

The two fractions in the relaxation curve of the water signal are clearly resolved, permitting the determination of all the parameters in equation (2). A fit to the data points (see Fig. 2) yields R_1^+ , R_1^- , c_w^+ and c_w^- ; using these values, R_{1w} is calculated, from equation (3d), to be $31 \pm 4 \text{ s}^{-1}$. A similar calculation for the collagen proton signal decay is less accurate, but gives $R_{1c} \approx 3 \text{ s}^{-1}$, which is similar to R_{1c} measured in D_2O . p_c and p_w can be determined from the relative signal intensities in the FID, extrapolated to zero time (Fig. 1), or from the known amounts of protons in the two phases. Within experimental error the two methods give the same values for p_c and p_w .

The parameters that describe the complete relaxation behaviour consistent with equations (3) are given in the legend to Fig. 2. For the cross relaxation rate k_c we obtain an estimate of $40 \pm 10 \text{ s}^{-1}$ for a hydration of 28 g H_2O per 100 g collagen. We believe that this is the first time that a figure has been given for the proton spin diffusion rate from a protein to the hydration water. Our measurements show that the water phase constitutes the relaxation 'sink' for the collagen proton phase. This result differs with the model of Kimmich and Noack¹⁻⁶, who proposed that the water relaxation proceeds via relaxation 'sinks' located with the macromolecular phase.

Usually, water relaxation rates in hydrated biological systems are obtained from plots similar to Fig. 2. It is common practice, however, to ignore, or avoid measurements (because of associated experimental difficulties) during the initial 10 ms of the relaxation decay. It is clear from our analysis that this practice would lead to a measurement of an apparent relaxation rate of water (R_1^- in the present case), which is not necessarily the same as R_{1w} . We find for the hydrated collagen sample in Fig. 2 that $R_{1w} \approx 2R_1^-$. Clearly, cross relaxation can significantly affect water proton spin-lattice relaxation in biological

samples. Its importance must be more carefully evaluated before applying molecular dynamical interpretations to apparent water relaxation rates. We are currently measuring cross relaxation effects in other hydrated biological systems and evaluating its consequences for a published analysis⁹ of the relaxation behaviour in hydrated collagen.

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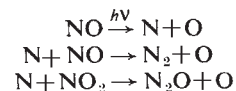
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Effect of NO photolysis on NO_y mixing ratios

It has been suggested that the only significant sink for odd nitrogen in the stratosphere is through transport of HNO_3 to the troposphere¹. As a result, if NO were to be injected in the middle or lower stratosphere, the change in odd nitrogen, NO_y ($NO_y \equiv NO + NO_2 + HNO_3 + N$), mixing ratio above the height of injection would be nearly constant. The photolysis of nitric oxide, however, and the subsequent reactions of nitrogen atom with nitric oxide act as a sink for odd nitrogens in the upper stratosphere. This mechanism has long been recognised as important to mesospheric chemistry^{2,3}, and has also been included in many of the stratospheric models¹⁻⁷. Its role in the stratospheric NO_y budget, however, has not been analysed. This paper reports an investigation on the importance of this net NO_y sink by means of a one-dimensional transport-kinetics model⁸.

In this model the reactions



provide a chemical sink for odd nitrogen in the upper stratosphere. We studied the effect of NO photolysis on NO_y mixing using a parameterisation of NO photolysis as shown by Cieslik and Nicolet⁹ and the chemical reactions used by Duewer *et al.*¹⁰. We found that inclusion of the sink would not result in a constant change in odd nitrogen mixing ratio above the height of a NO_x injection. This sink has a significant effect on model predicted effects of chlorofluoromethanes.

We computed an ambient NO_y mixing ratio and a perturbed NO_y mixing ratio due to an injection of NO at a rate of 2,000 molecules $cm^{-3} s^{-1}$ in a 1-km thick layer centred at 17 km using our standard chemistry (with the rate constant for $OH + HO_2 \rightarrow H_2O + O_2$ equal to $2 \times 10^{-11} cm^3 s^{-1}$). We then repeated the calculation with the rates of the NO photolysis sink set equal to zero.

Both of the above sets of calculations were repeated for the Hunten¹¹ eddy diffusion profile and for the Crutzen¹² eddy diffusion profile.

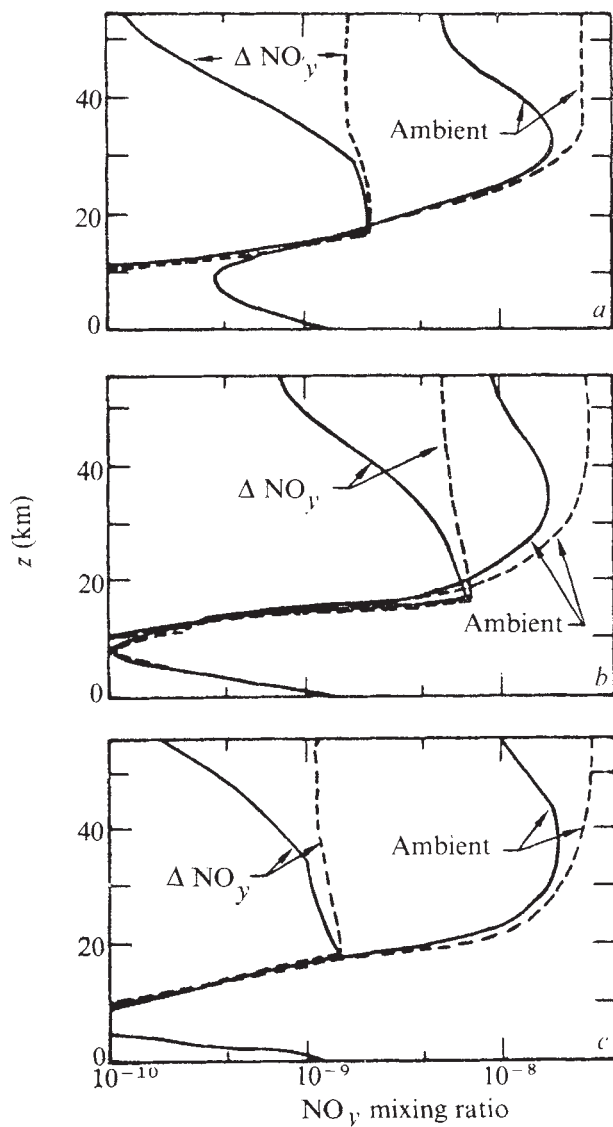


Fig. 1 Plots of computed ambient NO_y mixing ratios (ambient) and changes in NO_y mixing ratio after a 17-km NO_x injection (ΔNO_y) as described in text. —, normal model; ---, model with the NO photolysis sink suppressed. a, Chang⁸ K_z profile; b, Hunten¹¹ K_z profile; c, Crutzen¹² K_z profile.

The NO_y mixing ratios resulting from the above calculations are given in Fig. 1. A comparison of the results with and without the NO photolysis sink implies that this sink for NO_y is responsible for both decreasing (NO_y) at all altitudes and for a decrease in NO_y mixing ratio with altitude above about 30 km.

Note that for the perturbed cases the changes in mixing ratio are not constant with altitude even when the photolysis sink (the only chemical sink for NO_y in the model) is removed. This is a result of a change in the net rate of the 'natural' production of NO_y from the reaction



This source term changes because the ozone concentration

changes. The ozone reduction leads to a decrease in $\text{O}(^1\text{D})$ concentrations at high altitudes where the proportional change in solar flux is less than the change in ozone concentration, and an increase in $\text{O}(^1\text{D})$ at low altitudes, where the change in solar flux density more than compensates for the change in ozone. The changes in N_2O (because of altered solar flux) are relatively minor. The net change in the production of NO_y from $\text{O}(^1\text{D}) \cdot \text{N}_2\text{O}$ in the model with NO photolysis suppressed, is given in Fig. 2 for the three diffusion profiles.

The net changes in ozone column for the above calculations are given in Table 1. For ozone reductions, note that the rate coefficients used in the current calculations are the recommended values from Hampson and Garvin¹³, with $2 \cdot 10^{-11} \text{ cm}^3 \text{ s}^{-1}$ for $\text{OH} + \text{HO}_2$. Thus, there are numerous minor changes and one major change in the rate coefficients used here compared to those used to generate the CIAP findings¹⁴. Although omission of the N atom sink for NO_y makes a very substantial difference in the NO_y mixing ratio above 31 km, it causes only a minor increase in the percentage ozone perturbation for NO_x injections. This results because both the ambient and perturbed NO_y mixing ratios are reduced by the sink. The ozone perturbation is more strongly affected for the Hunten profile, which was used in the formula developed by NAS¹¹, than for the other diffusion profiles considered.

The effect of the N atom reactions on the O_3 response of the model to a ClO_x injection is opposite in sign and larger than their effect on the O_3 response to an NO injection. We find that, when the N atom sink is removed from our model, the stratosphere is substantially less sensitive to chlorofluorocarbons than when it

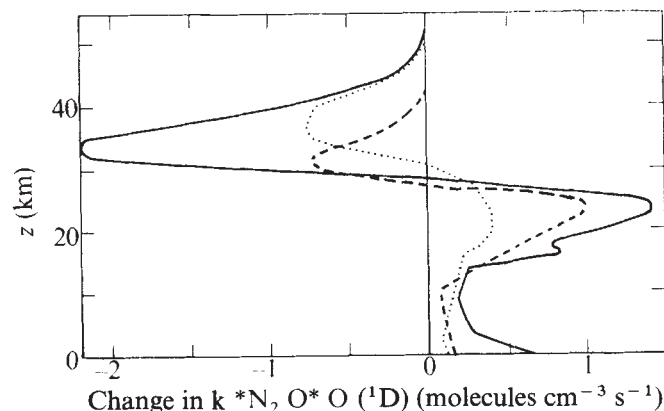
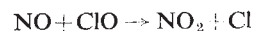


Fig. 2 Change in the odd nitrogen production rate in models with the NO photolysis sink suppressed. —, Hunten (1974) K_z profile; ---, Chang⁸ K_z profile; ····, Crutzen (1975) K_z profile.

is included. A model that computed a 0.51% reduction in ozone due to chlorofluorocarbons in 1976 using our full chemistry computed only a 0.30% ozone reduction in 1976 when NO photolysis was neglected. This effect occurs because the reaction



reduces the efficiency of both the chlorine and NO_x ozone destruction cycles, and the effect of this reaction is especially dependent on the NO_x mixing ratio above 35 km.

Table 1 Ozone columns (molecules cm^{-2}) and percentage deviations

	K_z (Chang)	K_z (Hunten)	K_z (Crutzen)
Normal, ambient O_3	7.4334×10^{18}	7.4627×10^{18}	7.4437×10^{18}
Normal, 17-km NO source	7.2920×10^{18}	6.9644×10^{18}	7.3459×10^{18}
ΔO_3 (%)	-1.75	-6.68	-1.31
No N atom ambient	7.2752×10^{18}	7.148×10^{18}	7.1165×10^{18}
No N 17-km NO source	7.1467×10^{18}	6.630×10^{18}	7.0195×10^{18}
ΔO_3 (%)	-1.77	-7.25	-1.36

Although models of the effects of NO_x injections on the stratosphere are not very sensitive to the NO photolysis sink for NO_x , inclusion of this sink is quite important for models of the effects of chlorofluorocarbons in the stratosphere. Indeed, the concentration of NO above 35 km is an important and poorly validated quantity in current models.

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Quantitative effects of sediment mixing on stratigraphy and biogeochemistry: a signal theory approach

SEDIMENT mixing by benthic fauna greatly affects the detailed historical record of the environment preserved in the sediments¹. Mechanical stirring is a major transport mechanism affecting fluxes of solid material and dissolved chemicals between sediments and the water column². Therefore, chemical and microbiological reaction rates in sediments are coupled to the rate at which substrate and product are transported to and from the site of reaction by bioturbation³. In order to understand better these processes, models which allow quantitative prediction of sediment mixing effects on inert and reactive sedimentary constituents are desirable.

The complexity of mixing events by benthic infauna has generally resulted in neglect of the environmental effects of mixing. Previous models have described mixing by a parametric 'diffusion coefficient'⁴ or 'mixing depth'^{5–8}, but such models have been applicable only under restricted assumptions of steady-state sedimentation and of constant and invariant mixing.

I have developed a general signal theory based model of bioturbation that does not require assumptions of temporal or spatial steady state, or of constant mixing to an invariant depth (Goreau, unpublished). This method allows calculation of the effect of any series of time-varying mixing events on any time-varying input to the sediment. It thus allows, when we have sufficient knowledge of the mixing history, the recovery of some original input information from sediments that have had their stratigraphic records mixed by bioturbation by comparison of actual profiles with model profiles based on realistic values of the relevant parameters.

As mixing moves material to shallower and deeper depths in sediments, corresponding to past and future inputs, there is no

unique age at each depth, but rather a mixture of material of different ages. It is useful to define a 'mixing function' $F(x)$, the depth profile of some unit quantity of inert material that has been introduced to the sediment surface at a discrete time and then been mixed and buried. Figure 1b shows the 'mixing functions' that result from the mixing and burial of three separate historical inputs to the sediment. If there were no mixing these would be found at depths X_0 , X_1 and X_2 . If we consider the profile that would result if some time-varying input was delivered to the sediments in the absence of mixing, $I(x)$ (Fig. 1a), then it can be shown that the mixed profile $O(x)$ (Fig. 1c) that results from the action of mixing on the input can be calculated as a convolution integral of input $I(x)$ and the mixing function $F(x)$:

$$O(x) = \int_{-\infty}^0 I(x') F(x - x') dx' \quad (1)$$

where x' is a dummy variable and mixing is represented as spatial filtering of the undisturbed input by the mixing function $F(x, x')$. The mixing process acts as a low pass frequency filter, allowing slow changes in input to be recorded in the sediment while attenuating rapid changes. Fine structure of the stratigraphic record is smeared out in a predictable manner, and recent events are recorded deeper in the sediment than they would be in the absence of mixing (Fig. 1d). As a result, estimates of sedimentation rates and palaeo-oceanographic changes derived from penetration depths of such recent inputs as the bomb-produced radionuclides ^{14}C and ^{137}Cs are often too high. The true value can be found if $F(x)$ is known.

$F(x)$ can be determined from measurements of $O(x)$, the natural mixed profile of a non-reactive constituent with known inputs, and measured directly when that input is a spike such as thin ash layers, microtektites, or rapid microfossil changes. When $F(x)$ is known at a particular site, it is applicable to other inert inputs, and can be used in conjunction with measured mixed profiles to deconvolve the effects of mixing by inversion of equation (1) to derive the original input history.

A similar mixing function is derived for pore water, $F_w(x)$, the volume of pore water exchanged with overlying seawater per unit area per unit time, as a function of depth (Fig. 1e). If the concentration of dissolved constituent with depth is $C(x)$ (Fig. 1f), and C_w in seawater, then the flux of the constituent across the sediment–water interface is shown to be

$$\text{Flux (g cm}^{-2} \text{ s}^{-1}) = \int_0^{x_{\max}} F_w(x) (C(x) - C_w) dx \quad (2)$$

When $C(x)$ exceeds C_w the flux will be from sediment to the water column, and when $C(x)$ is less than C_w the net flux will be into the sediments. This expression contrasts with Fickian diffusion terms used to estimate fluxes in previous work⁹, since bioturbation is much faster than molecular diffusion. Ions transported across the interface have their source or sink throughout the mixed zone of sediment, and are not just passively driven by diffusion along pore water concentration gradients at the interface, as assumed in diffusive flux calculations. Measured values of $F_w(x)$ may substantially modify estimated sediment–water geochemical fluxes.

Reactive inputs, consumed or produced in sediment, will have different depth profiles than inert inputs. The difference between mixed profiles resulting from similar reactive and conservative inputs is a measure of net consumption or production of inputs with depth. By interpreting carefully controlled laboratory experiments in conjunction with the present approach, I believe it possible to estimate means and ranges of net reaction rates as a function of depth in naturally mixed sediments to obtain quantitative measures of geochemical and microbially mediated reaction rates in sediment. These rates should be quite different from those estimated in the absence of mixing, since redox potential dependent reactions are affected by the rate of oxygen

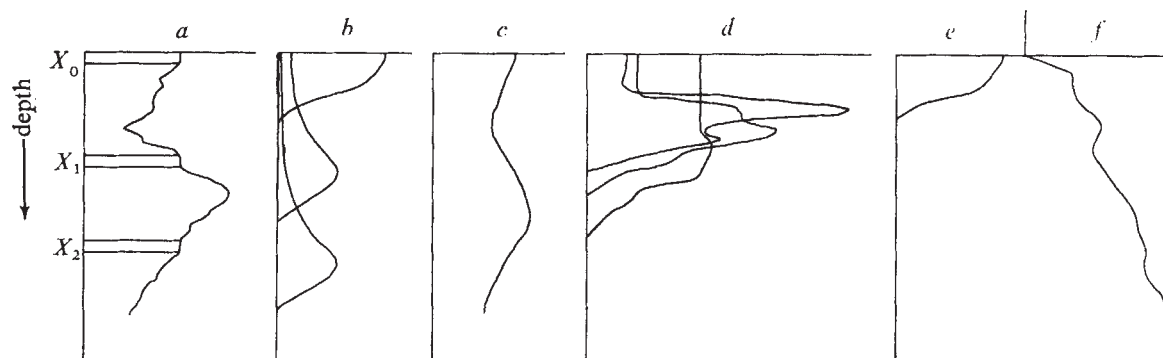


Fig. 1 *a*, An undisturbed time varying sedimentary input, $I(x)$, that would be observed in the complete absence of mixing. *b*, Mixing functions showing the depth profiles caused by the mixing and burial of three successive inputs, $I(x)$, $I(x_1)$ and $I(x_2)$. *c*, $O(x)$, the mixed output obtained by operation of the mixing function on $I(x)$. The mixed output is derived from the time-varying input and mixing function through the finite difference form of equation (1): $O(x) = \sum_i I(x_i) F(x, x_i)$. *d*, Calculated effects of uniform mixing to 1, 2 and 4 relative depth units on the fallout inputs of the bomb-produced radionuclide ^{137}Cs . (Increased depth of mixing is seen to push the ^{137}Cs maximum deeper into the sediment and obliterate fine scale information in the mixed profile.) The calculated profiles are similar to those measured by Pennington *et al.*¹⁰. This choice of mixing functions produces results similar to those of Robbins and Edgington¹¹. *e*, $F_w(x)$, the volume of pore water exchanged with overlying water per unit area per unit time, as a function of depth, due to bioturbational mixing of sediment and faunal water pumping. *f*, A typical depth profile of a dissolved nutrient such as ammonia or phosphate in pore water. The flux of the dissolved constituent across the sediment–water interface is given by equation (2).

transport into sediment. Bioturbation affects oxygen fluxes, the Eh microhabitat, and ecological depth zonation of the microfauna that mediate such environmentally important processes as aerobic heterotrophic decomposition, sulphate and nitrate reduction, and ammonia and methane generation.

This work shows that many stratigraphic, geochemical and microbiological problems in recent sediment can be approached through measurable 'mixing functions' characterising the real-world dynamic effect of mixing in a quantitative and predictively useful manner. Further development of the numerical model with applications to the spatial and frequency response of the historical record, detailed stratigraphic records of bomb-produced radionuclides, the deconvolution of recent pollutant trace-metal sediment profiles, sediment–water fluxes, temporal and spatial variation, and the estimation of sediment geochemical and microbiological reaction rates will be published elsewhere.

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Evidence of major sulphate evaporite deposits in the Proterozoic McArthur Group, Northern Territory, Australia

WE report the occurrence of extensive sulphate evaporite relicts from the McArthur Group (1,600–1,400 Myr old), Northern Territory (16° 30' S: 135° 30' E approx), Australia. The McArthur Group comprises a sequence of carbonates, shales, siltstones and sandstones, with a minor volcanic

component, of mid-Proterozoic sediments and includes the huge H.Y.C. stratiform Pb–Zn–Ag deposit¹.

Some models for the early evolution of the Earth's hydrosphere² postulate that sulphate evaporite minerals were not deposited before late Proterozoic times (~800 Myr ago)^{3–7}. However, our work, and other evidence^{8–11} indicates that these evaporite minerals do indeed occur in older rocks.

Pseudomorphs after halite and gypsum were first reported from the Group in the 1960s^{12–14}. Our present studies show that these and other evaporite relicts are much more widespread and significant than was originally thought. Most of the evaporite relicts are in the form of carbonate pseudomorphs after a variety of morphologies of gypsum and anhydrite crystals, chert pseudomorphs after anhydrite nodules (=the 'botryoidal quartz' of ref. 5), halite casts, and microscopic remnants of original, unaltered sulphate minerals. The characteristics of each of these evaporite relicts are described and their distribution within the McArthur Group is shown in Table 1.

The most abundant evaporite relicts in the McArthur Group are carbonate pseudomorphs after discoidal and euhedral to subhedral gypsum and anhydrite crystals (Fig. 1a). The size of the pseudomorphs is variable. The discoidal crystals range in length from less than 1 cm to about 15 cm. They are lensoid in cross section, with length to breadth ratios varying from about 2:1 to 15:1. The euhedral pseudomorphs are less common than the discoidal ones and range in length from about 5 mm to 3 cm. We have found discoidal pseudomorphs in quartz arenites, and they are very abundant in a number of carbonate lithologies.

In quartz arenites, the pseudomorphs are disseminated and randomly oriented, and they contain numerous small inclusions of quartz and dolomite. The distribution of the pseudomorphs in the carbonate rocks, however, varies from a few randomly oriented and disseminated specimens, to whole beds of pseudomorphs (originally mapped as sideritic marbles¹²), ranging from 10 cm to 45 cm thick. In the massive beds, discontinuous wisps of chert characteristically delineate algal mats, domal stromatolites and *Conophyton*. In the carbonate rocks, pseudomorphs cross cut sedimentary features such as bedding and laminated algal mats, suggesting that the original sulphate minerals crystallised in the host sediments during diagenesis. The pseudomorphs may be either single crystals or mosaics of anhedral grains displaying a polygonal texture. The grains vary in diameter from 50 to 100 μm , and are generally larger than the grain

size of the host sediment. As well as carbonate, the pseudomorphs frequently contain traces of disseminated pyrite, and occasional blebs of chalcedonic quartz. Electron probe microanalyses of the carbonate minerals show that they are iron-rich phases such as ferroan dolomite, ferroan magnesite (bruenerite) and magnesian siderite (pistomesite), whereas the host carbonate is usually less iron-rich dolomite or magnesite. Some pseudomorphs are compositionally zoned with respect to their iron content. In some of the pseudomorphs, we have observed tiny, highly birefringent inclusions, reaching a maximum size of 10–20 μm , and have identified them as anhydrite, using the petrological microscope. Our identification was confirmed by electron probe microanalysis. Using these techniques, we have also identified nodules of anhydrite averaging 2–3 mm in diameter from a silicified algal mat horizon in a massive pseudomorph bed in the Amelia Dolomite.

The discoidal pseudomorphs are identical in habit with the gypsum which grows interstitially in Recent and ancient sabkha and intertidal environments^{15–18}. Both have curved faces and both are frequently flattened in a plane perpendicular to the *c* crystallographic axis. Others, however, have spear shaped or serrated terminations that possibly reflect repeated twinning of the original crystals (Fig. 1*b*). Such forms are also characteristic of anhydrite crystals that are found in Recent and ancient marginal sabkhas^{17–19}.

Apart from the discoidal and euhedral pseudomorphs described, the McArthur Group also contains acicular car-

bonate pseudomorphs. These were tentatively identified as being dolomite pseudomorphs after aragonite²⁰, and were believed to be restricted to the Coxco Dolomite Member of the Teena Dolomite (Table 1). Our studies, however, show that as well as the Coxco Dolomite occurrence, acicular pseudomorphs are also present in the Emmerugga Dolomite. The needles are six-sided, and their interfacial angles are the same as those of gypsum crystals elongated parallel to the *c* crystallographic axis (see Fig. 2). The acicular pseudomorphs vary from 2 to 10 cm in length, and from 1 to 5 mm in diameter, and they occur in radiating clusters. In thin section, the needles are seen to be composed of mosaics of anhedral carbonate grains with an average diameter of 100 μm . The pseudomorphs are zoned, having rims of clear dolomite, with cores of dark dolomite containing abundant fine-grained inclusions of what appear to be clay minerals. The carbonate mineral in the pseudomorphs is ferroan dolomite identical in composition with the host dolomite.

The acicular pseudomorphs are identical in habit to a variety of gypsum forming in shallow brine pools on the sabkhas of the Trucial Coast (D. Kinsman, private communication). According to Kinsman, the root of each cluster is situated at the sediment–water interface. The larger acicular gypsum needles are often hollow and partly filled with fine-grained detritus. We therefore interpret the zoning of the McArthur acicular pseudomorphs to have been formed by similar trapping of fine-grained detritus in once hollow gypsum needles, although they may have been

Fig. 1 *a*, Distribution of carbonate pseudomorphs in carbonate beds. (Scale bar : 0.5 cm.) *b*, Evaporite pseudomorphs with serrated terminations. (Scale bar : 0.5 cm.) *c*, Botryoidal quartz nodule. *d*, Thin section of botryoidal quartz, showing lutecite crystals. (Scale bar : 0.5 mm.)

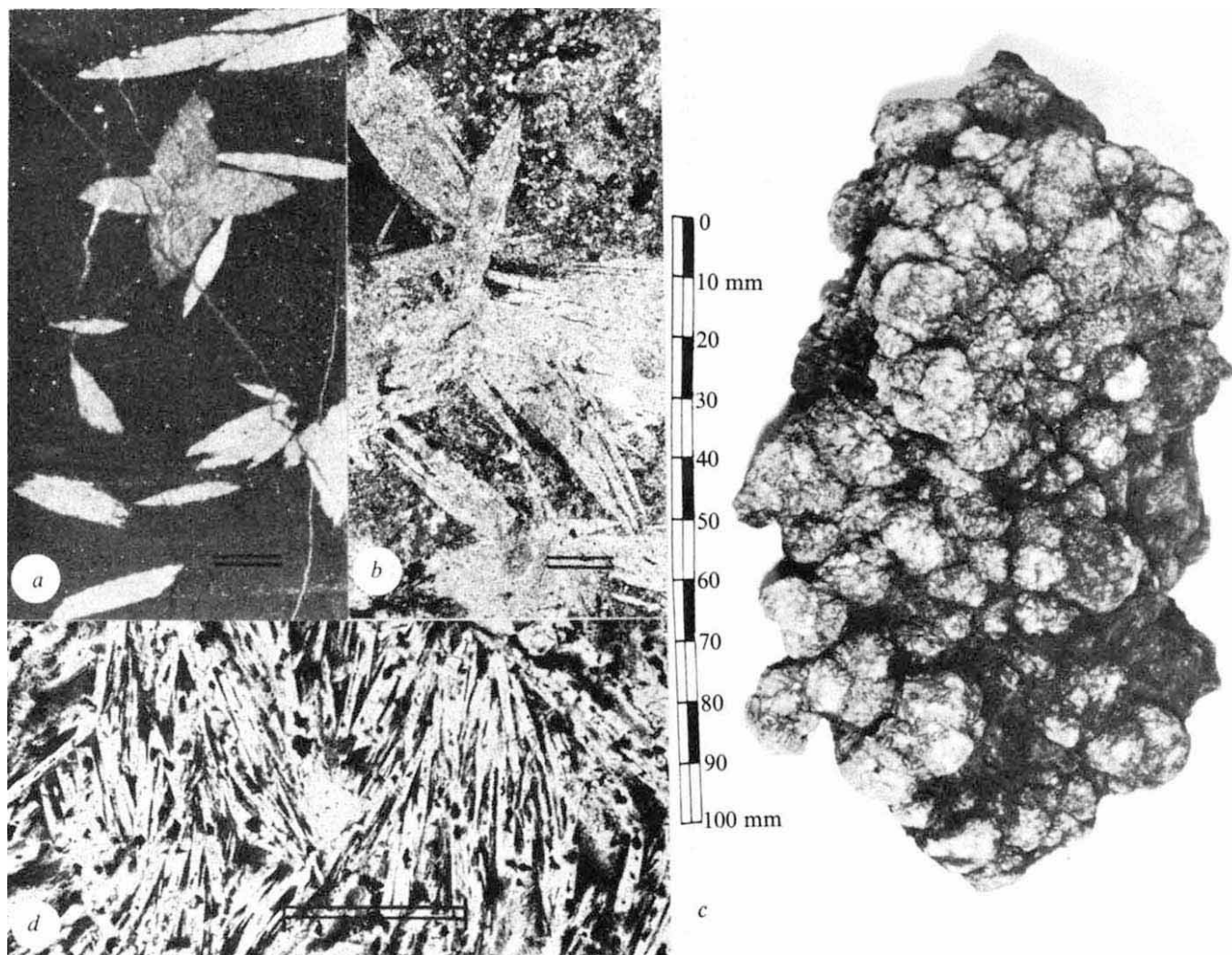


Table 1 Regional stratigraphy and distribution of evaporites, in the McArthur Group. H, halite casts; LD, euhedral and discoidal gypsum and/or anhydrite pseudomorphs; N, acicular gypsum pseudomorphs; C, cauliflower chert nodules

	Formation	Main lithologies	Thickness (m)	Evaporite pseudomorphs
Batten Sub-group	Stott Formation	Massive and stromatolitic dolomite; dolomitic shale	750	LD
	Smythe Sandstone	Quartz sandstone; chert conglomerate	0-180	
	Looking Glass Formation	Chert; chert breccia; minor dolomite	25-230	
	Stretton Sandstone	Flaggy quartz sandstone	30-245	
	Yalco Formation	Chert; chert breccia; minor dolomite	130	
Umbolooga Sub-group	Lynott Formation	Dolomitic siltstone; chert; chert breccia; minor dolomite	210-760	LD, C (Donnegan Member)
	Reward Dolomite	Dolomite with chert pellets; dolomitic siltstone	50-300	
	Barney Creek Formation	Pyritic, carbonaceous, dolomitic shale; dolomite; dolomite breccia; H.Y.C. deposit	0-490	
	Teena Dolomite	Massive laminated and stromatolitic dolomite	57	H, N (Coxco Dolomite Member)
	Emmerugga Dolomite	Massive laminated and stromatolitic dolomite	300	H, LD, N
	Tooganinie Formation	Stromatolitic dolomite; dolomitic shale; quartz arenite, dolomitic siltstone	800	H, LD
	Tatoola Sandstone	Quartz arenite; dolomitic siltstone	140	LD
	Amelia Dolomite	Laminated and stromatolitic dolomite, massive gypsum pseudomorphs in beds	90-240	H, LD, C
	Mallapunyah Formation	Purple dolomitic siltstone; minor dolomite; quartz arenite	30-750	H, LD, C

formed by a mechanism similar to that suggested by Kastner²¹.

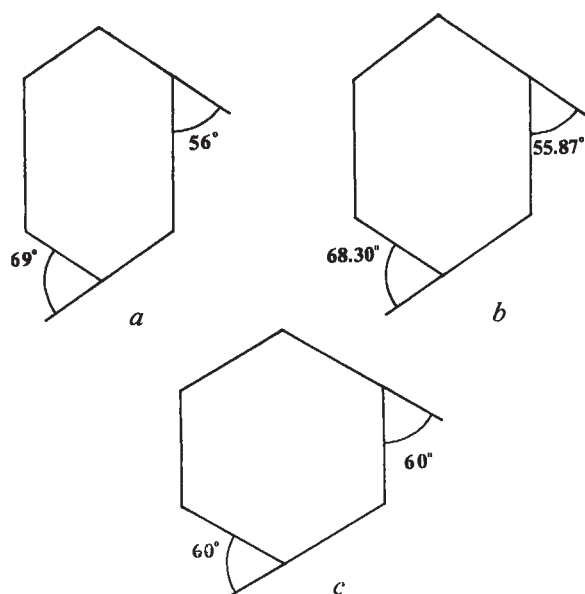
'Cauliflower' chert nodules²³ also occur in the McArthur Group, and are particularly abundant in the Mallapunyah Formation, and in the Donnegan Member of the Lynott Formation (Table 1). The nodules are roughly circular in plan and are flattened parallel to the bedding. They vary from about 1 to 100 cm in diameter, and from 1 to 30 cm in height. The upper surfaces of the nodules are irregular and botryoidal (Fig. 1c). The lower surfaces are similar but more flattened. The outer surfaces have a well developed mesh-like structure of interlocking chert blades. In thin section three zones can be recognised. The outer zone consists of a mesh of lutecite fibres²² set in a matrix of brown microcrystalline quartz (Fig. 1d). In some cases, the fibres display a distinct, sub-parallel lineation, and they contain highly birefringent inclusions which we have identified as anhydrite. This outer zone grades inwards to a zone of

macrocrystalline quartz that contains minor amounts of dolomite and barite. The innermost zone of the nodules consists of coarsely crystalline dolomite and barite, together with trace amounts of fluorite. These nodules are remarkably similar to the chert nodules from the Fort Payne and 'Warsaw' Formations of Tennessee which Chowns and Elkins²³ demonstrated to be replacements of anhydrite nodules identical with those described by Kinsman from the sabkha environments of the Persian Gulf¹⁹.

Pseudomorphs after halite are common throughout the McArthur Group with the highest concentration in red beds and some dolomitic siltstone sequences (Table 1). Brown and Plumb²⁴ describe the characteristics of the halite casts in the Group, and the present studies have produced little new information. The halite casts which we have seen up to now, all appear to have formed by almost complete evaporation in shallow marine environments, and probably represent ephemeral salt crusts. Pseudomorphs after halite coexisting with either gypsum or anhydrite are extremely rare. This general lack of association of halite and calcium sulphate minerals probably results from the dissolution of previously deposited halite during surface flooding, as it does in Recent marginal sabkhas^{17,18}.

While we cannot determine precisely the amount of evaporite material because of poor outcrop in the McArthur River area, our studies show that evaporitic sediments occur over an area of at least 5,000 km² in the Batten Trough alone²⁵. In some sections of the particularly evaporitic formations, for example, the Amelia Dolomite, up to 40% of the measured sections are composed of evaporite relicts. Therefore, not only do our studies show that a variety of evaporite relicts occur in the McArthur Group, but that they occur in vast amounts.

Although our work is still at an early stage, the results obtained so far lead us to several important conclusions. First, we conclude that significant parts of the McArthur Group were deposited in a marginal sabkha environment. Many of the evaporite sequences of the McArthur Group are remarkably similar to the Recent sediments of the Trucial Coast. This resemblance provides the basis for new insights into the sedimentary environments represented in the McArthur Group, the habitats of the microfossils which abound in many of the formations²⁶⁻²⁸, and suggest possible

Fig. 2 Interfacial angles of pseudomorphs in radiating clusters (a) compared with interfacial angles of gypsum (b) and aragonite (c).

physiographic configurations and climatic regimes. Second, the McArthur Group evaporites which are similar to Recent coastal sabkha deposits in terms of chemistry, crystallographic habit etc., are probably the products of brines with essentially the same compositions. Third, the Persian Gulf sabkha model is not applicable to all of the McArthur Group evaporites, some of which appear to have stronger resemblances to the Marion Lake, Yorke Peninsula (South Australia) lacustrine gypsum deposits²⁹. Fourthly, our results show conclusively that the giant H.Y.C. stratiform Pb–Zn–Ag deposit at McArthur River is yet another example of the widespread association of stratiform base metal sulphide deposits with sulphate evaporitic sediments^{30–32}. The location of the H.Y.C. deposit within a large evaporite bearing basin raises the interesting possibility that this and related deposits formed from metal-rich brines of the kind forming today in evaporite-bearing basins³³, rather than from volcanically derived hydrothermal solutions³⁴.

Finally, we maintain that the evidence presented here and elsewhere¹ unequivocally shows the widespread occurrence of large volumes of sulphate evaporites in mid-Proterozoic times. The assertion that there was no significant deposition of such evaporites before ~800 Myr ago² is manifestly in error. Consequently, the models of crustal, hydrospheric and atmospheric evolution³ based on this contention require considerable revision and modification.

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Discovery of amphibians in the Namurian (Upper Carboniferous) of Fife

CARBONIFEROUS amphibians are very rare as fossils and almost all major finds of British specimens were made in the nineteenth century. Nevertheless, knowledge of Carboniferous tetrapods, both amphibians and reptiles, has expanded considerably in recent years^{1,2}. All are from Europe or North America and most are from horizons equivalent to or later than the British Coal Measures (Westphalian and Stephanian, respectively). Far fewer amphibians are known from the Lower Carboniferous and knowledge of the Namurian tetrapod fauna, between the Lower Carboniferous and the Coal Measures, is even more meagre. With the exception of a single incomplete anthracosaur amphibian from the Namurian of the Ruhr, Germany³, pre-Coal Measure amphibians are known from only three areas (1) West Virginia and south-western Pennsylvania in the United States^{4,5}; (2) Nova Scotia, Canada⁶, and (3) the Edinburgh area and southern Fife in Scotland^{7,8}. In November 1974, however, fossil amphibian and fish remains were discovered at the Dora Opencast Site (area C), south-east of Cowdenbeath, Fife (Fig. 1), in Carboniferous strata of Namurian age. The richly fossiliferous bone bed at Cowdenbeath formed a localised patch in a seatrock (rooty, muddy siltstone), which lies beneath a coal seam below the Lochgelly Blackband Ironstone. The Lochgelly Blackband Ironstone itself has also produced a fauna with amphibian remains in the Dora site, while a few specimens have been recovered from strata up to 6 m lower in the sequence.

All these strata belong to the Limestone Coal Group which forms the lowest subdivision of the Namurian Series (lower Namurian A) in Scotland. In the Dora area this subdivision is about 330 m thick. A measured section (Fig. 2) at the locality indicates the position of the bone bed about 62 m beneath the base of the Index Limestone at the top of the Limestone Coal Group. Currie⁹ took the base of the Index Limestone as the junction between the top of the Pendleian (E1) and the base of the Arnsbergian (E2) stages, a conclusion which is supported by Wilson (ref. 10, Fig. 1).

The bone bed was originally exposed as a section some 30 m long, but thanks to the generosity of the Murphy Group, contractors to the National Coal Board, the considerable overburden was removed to expose the upper surface of the bone bed, which dipped gently north-westwards. The bed was cut off by a fault at the south-west end and divided by two small faults into three areas. Three 1.25-inch (31-mm) diameter cores were taken from the surface of the bed to a maximum depth of 5 m, by K. Hall and S. Creany

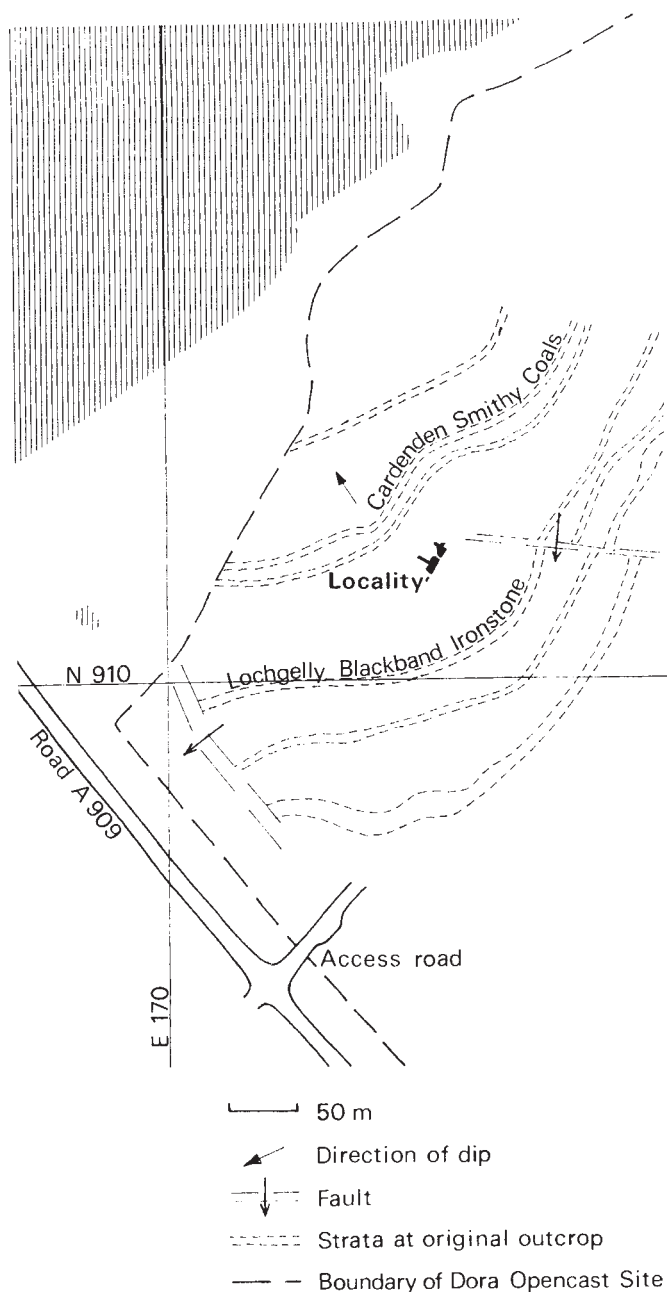


Fig. 1 Position of the fossil amphibian locality in Dora Opencast Coal Site, south-east of Cowdenbeath, Fife (NT 172911). From National Coal Board Opencast Executive Scottish Region, Drawing No. 01/6028.

of the Organic Geochemistry Unit, University of Newcastle upon Tyne. After gridding into 0.33-mm squares to preserve relationships between associated remains, the richest part of the bone bed was collected by hand. This material, together with transects extending outside the richly fossiliferous area, is stored in the University of Newcastle upon Tyne, in the Royal Scottish Museum and by S. P. Wood. After a delay from October to December 1975 to facilitate collection, opencast operations have removed all the surrounding and underlying strata.

Several specimens have already been developed from the bone bed. Many of these are isolated skeletal elements of sharks and of representatives of all the major groups of bony fish known from the Carboniferous. These include acanthodians, actinopterygians, dipnoans (lungfish) and crossopterygians, the group from which the tetrapods are agreed to have descended. The acanthodian fish *Gyracanthus* is by far the commonest form, represented in nearly

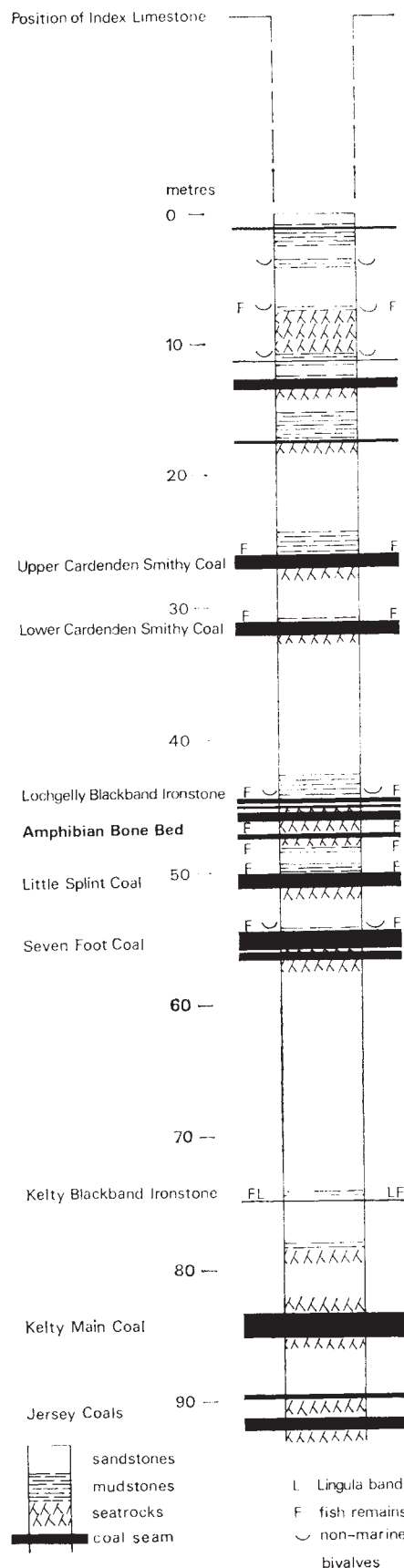


Fig. 2 Composite measured section in Dora Opencast Site (NCB), areas C and D (Institute of Geological Sciences registered numbers NT 19 SE/534 and 537).

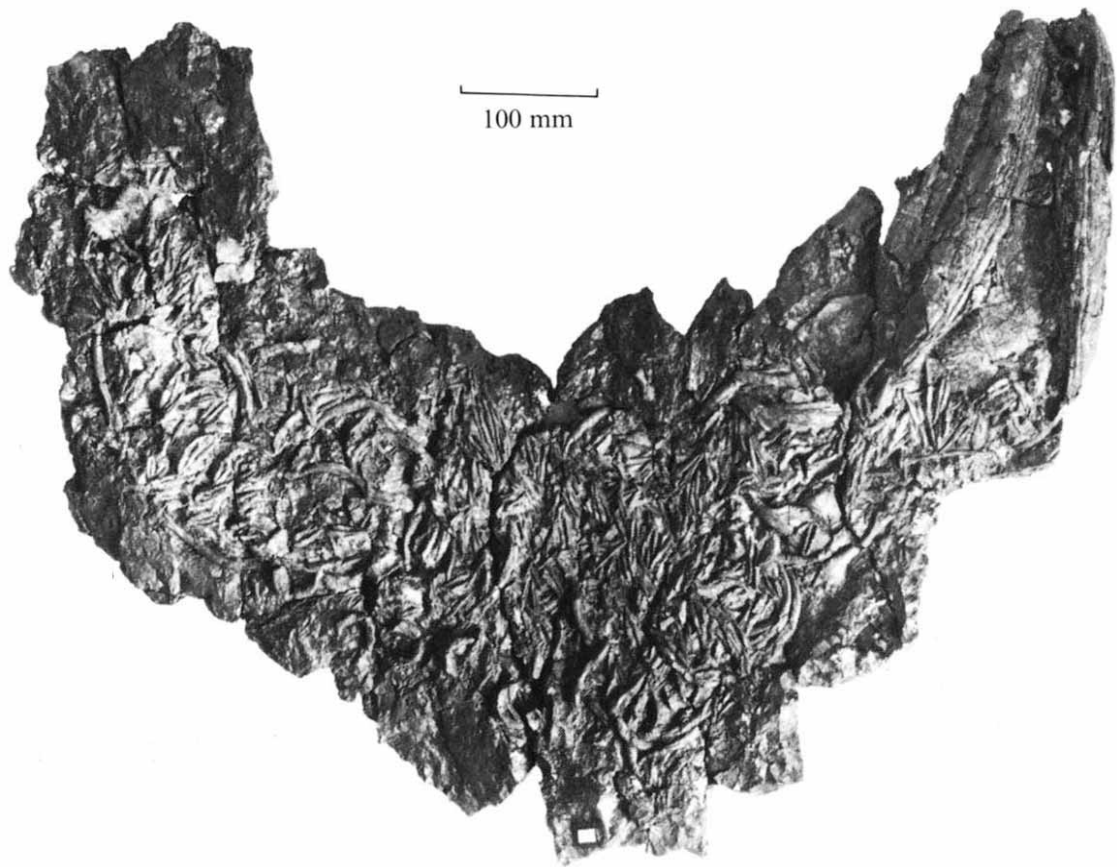


Fig. 3 Skeleton of a new anthracosaur amphibian (BM(NH)R 10,000) in ventral view.

every 0.33-m block of matrix by at least one spine, whereas the actinopterygians are represented only by a single specimen showing palaeoniscid scales. The skull bones of rhizodontiform crossopterygians will give important information on this poorly known group. The fish fauna of the Lochgelly Blackband Ironstone appears to be similar to that of the bone bed but includes six determinable actinopterygian species.

The amphibian specimens include representatives of all three major Palaeozoic groups, the Anthracosauria, Temnospondyli and Lepspondyli. By far the most important specimen so far developed is the almost complete but tail-less specimen of a new crocodile-like anthracosaur amphibian, now registered in the British Museum (Natural History) (Fig. 3). Skull remains of a second anthracosaur are probably to be referred to a primitive Lower Carboniferous species, *Eoherpeton watsoni* Panchen² and a third anthracosaur, as yet undescribed, is represented by the skull table and interorbital region (Fig. 4). Temnospondyls include a jaw ramus of the aberrant loxommatid *Spathicephalus mirus* Watson⁷, previously known only from a similar horizon at Loanhead near Edinburgh, and appendicular bones probably to be referred to the primitive long-bodied Colosteidae². The small lepospondyl amphibia are represented by isolated postcranial remains.

The Carboniferous was the period of the first major adaptive radiation of tetrapod vertebrates and the earliest reptile known is from the early Coal Measures (Westphalian B) of Nova Scotia¹¹. The Cowdenbeath find could extend reptile history down into the Namurian. It also has the potential to vastly increase knowledge of the early Carboniferous fauna beyond the small number of species and individuals from other early Carboniferous sites. Furthermore, the Namurian fauna is particularly important in forging the evolutionary link between the few primitive amphibia known from the Lower Carboniferous and the more advanced and diverse fauna of the Coal Measures.

Study of the Cowdenbeath collection is in progress at the Royal Scottish Museum and, supported by a grant from NERC, in the University of Newcastle upon Tyne. We thank Dr F. J. Matheson and Mr D. J. Harley, National Coal Board, Opencast Executive, for permission to collect and Messrs A. P. Duffy and K. D. Smith of the Murphy Group

Fig. 4 Skull table of a new anthracosaur amphibian, ? *Embolomeri*.



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Earliest known *Hipparion* from Holarctica

THE three-toed horse *Hipparion* was ubiquitous in terrestrial mammalian faunas throughout Holarctica during parts of the Miocene. This report describes *Hipparion* from the early Barstovian Fleming Formation of the Texas Gulf Coastal Plain. These specimens are assigned to *Hipparion* because of dental characters such as the diagnostic presence of isolated protocones in the upper molars. This *Hipparion* is also characterised by a well-developed and anteriorly-rimmed preorbital fossa similar to the condition seen in some Old World forms. Because of this earliest known occurrence, the widely used *Hipparion* datum concept should be restricted to some palaeogeographically finite area in the Old World.

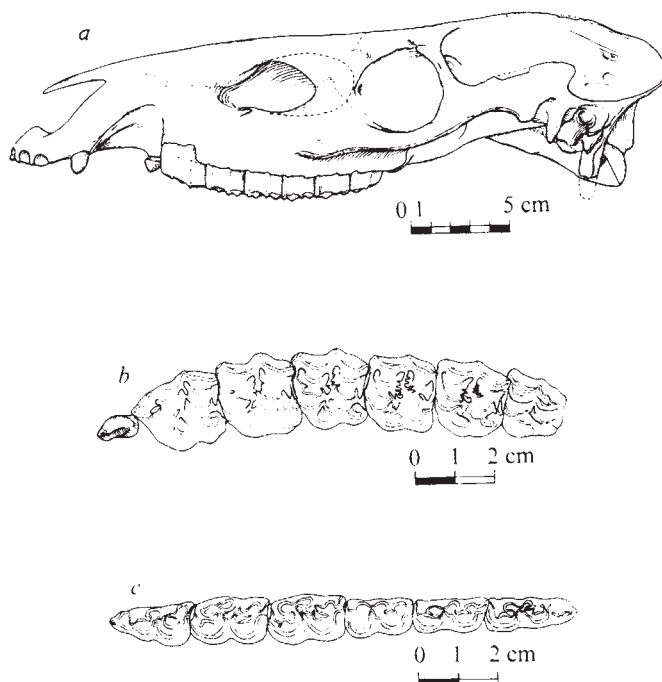
We prefer the designation '*Hipparion*' in the broad sense because we contend that, in common usage, it represents a 'form genus' consisting of Miocene-Pliocene horses with isolated protocones in the upper molars and tridactyl feet, that is, *Hipparion* is not used in the strict taxonomic sense. Because of its great abundance both geologically and geographically, this horse has played a significant role in the biochronology of this time interval, with Eurasian Neogene mammalian assemblages often being termed *Hipparion* faunas. The first appearance of *Hipparion* has been used as a geologically synchronous time plane, also termed 'datum', much in the same way as microfossils have been used in marine sequences. Based on a combination of different geochronological techniques, the *Hipparion* datum has been determined to be 12.5 Myr in the western Old World^{1,2}, 12.8 ± 1.03 Myr in northwestern Africa³, and 12–9 Myr in Sub-Saharan Africa⁴. In the well known mammal-bearing Siwalik sequence of the Sub-Himalayan region, the *Hipparion* datum is above the base of the Nagri Sandstone and *Hipparion* is common in the middle Siwaliks^{5–7}. Precise determinations for this datum are not available. *Hipparion* is also abundant from parts of the

Nearctic Miocene. Until now, the first occurrence of this horse in the New World has been thought to be of early Clarendonian age.

In the collection at the American Museum of Natural History, New York, are several skulls and dentitions of *Hipparion* from Trinity River Pit 1, early Barstovian age (medial Miocene), Fleming Formation, San Jacinto County, Texas Gulf Coastal Plain (Fig. 1). There is no direct geochronological control for this locality. Based on correlation to other early Barstovian assemblages with radiometric age determinations, the Trinity River Pit 1 seems to be significantly older than the Old World localities in which *Hipparion* first occurs, that is of the order of approximately 15 Myr (ref. 8). These specimens belong to a distinctive group of *Hipparion*, also represented in the Old World, that have a well-developed and anteriorly-rimmed preorbital fossa. A detailed discussion of the taxonomic significance of preorbital fossae in *Hipparion* is the subject of another report⁹. The dentition is brachyodont relative to other *Hipparion*. The upper molars are characterised by the distinctive *Hipparion* isolated protocone, which in the sample from Trinity River Pit 1 is rounded in cross section. The plications on the fossette borders are simple relative to other *Hipparion*. The lower cheek teeth have separated metaconids and metastylids and either deep externomedial valleys (ectoflexids) or weak plicaballinids.

It is generally accepted that *Hipparion* arose in the New World during the Miocene from some species of *Merychippus* and subsequently dispersed to the Old World^{10,11}. The *Hipparion* from the early Barstovian of Texas represents the earliest-known member of this group. It is primitive in several aspects, including small size, rounded protocone, and brachyodontology, and advanced in the development of the diagnostic anteriorly-rimmed preorbital fossa, which is also found in later representatives of this group⁹. In younger Clarendonian sediments of the New World,

Fig. 1 Earliest known *Hipparion*, from Trinity River Pit 1, Fleming Formation, early Barstovian, San Jacinto County, Texas Gulf Coastal Plain. a, F:AM 73940, skull that shows pre-orbital fossa. b, F:AM 73940, left upper cheek teeth. c, F:AM 73940, left lower cheek teeth. F:AM is the abbreviation for; Frick: American Mammals, The American Museum of Natural History.



Hipparion is represented by a few morphologically distinct groups that can be characterised by differences in the development of preorbital fossae, and, to a lesser extent, differences in dental patterns. In the Neogene of the Old World, *Hipparion* first appears at the base of the Vallesian, which represents in part the same time interval as the Clarendonian of the New World². The presence of *Hipparion* in the Old World seems to represent dispersal from the New World. Based on characters of the dentition, it has been argued that *Hipparion* in the Vallesian of the Old World was monophyletically represented by one species, *H. primigenium*. It has also been hypothesised that *Hipparion* underwent an adaptive radiation during Vallesian and later time that resulted in the multitude of species described in the literature¹². But, based on differences in preorbital fossae in Holarctic *Hipparion* we suggest that clearly differentiated forms of *Hipparion* were already in existence in the New World by Clarendonian time, and dispersal to the Old World could have been polyphyletic with the presence of several forms during Vallesian and later time.

Some early workers used the first appearance of *Hipparion* in Holarctica as the herald of the Pliocene^{13,14}. With the advent of more recent biostratigraphic and geochronologic interpretations, this hypothesis is no longer considered valid by most workers^{1,2}. Although the *Hipparion* datum does not correspond to the Mio-Pliocene boundary, it is still used as a geologically synchronous event for purposes of correlation in the western Old World. The presence of *Hipparion* in early Barstovian sediments of the New World is significantly older than the first appearance of *Hipparion* in the Vallesian of the western Old World. The question now arises, to what palaeogeographic extent should the *Hipparion* datum be restricted in order to retain its usefulness as a geologically synchronous event? Much evidence has been presented to demonstrate the usefulness of the *Hipparion* datum in the western Old World and Africa. Precise geochronological studies are needed to determine when and what form(s) of *Hipparion* first appeared in the eastern Old World. If the first appearance of *Hipparion* is not a geologically synchronous event throughout the Old World, or if it represents dispersal of several forms, then it would not be necessary to reject the *Hipparion* datum concept, but it would have to be restricted to some palaeogeographically finite area.

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Tail autotomy, functional conflicts and their resolution by a salamander

If an organ serves several functions, two or more of these may conflict at times in their contribution to the immediate fitness of the organism. Among lower invertebrates the tail is commonly used as a defence against predators but it has other functions such as fat storage, locomotion, respiration and courtship. Experiments have shown that tail loss can depress reproductive output in lizards^{1,2}, but this has not been reported for natural populations. Data reported here suggest that loss of the tail frequently depresses reproductive output in natural populations of the plethodontid salamander *Batrachoseps attenuatus*, which is widespread in California. The dual use of the tail for energy storage and for protection may increase the adaptive flexibility of the salamander for coping with an unpredictable environment.

B. attenuatus has a tail which may reach twice the trunk length. It commonly represents 50% of the biomass of mature animals, and can provide proteins and lipids for maintenance, reproduction and continued growth in trunk length. During the dry summer months *B. attenuatus* retreats underground and does little or no feeding³. At this time females produce eggs and males come into full breeding conditions (large premaxillary teeth develop and neck muscles enlarge). During the dry season, the mean tail volume of mature individuals has decreased by 29%. Thus the tail is presumably an important energy source for maintenance and reproduction during this period.

Tail autotomy seems to be an adaptation for escaping from predators. It has been associated with attacks from snake predators in the laboratory (my unpublished results and personal communications from S. Arnold). Although autotomy may be associated with intraspecific competition, I have seen no evidence for this during experiments on competition for burrow sites. The tail of *B. attenuatus* can drop off at any segment; it is then regenerated at apparently a constant rate until it approaches its full length⁴. Estimates of annual tail breakage in populations in or near Berkeley, California, range from 28 to 48% (data to be published elsewhere). For only half the time, however, does a salamander lose more than 30% of its tail. Size and sex classes do not differ significantly in the frequency of observed tail breaks.

Three lines of evidence suggest that tail autotomy inhibits reproduction in natural populations of this salamander. First, mature individuals, both males and females, which fail to breed during one season have significantly shorter tails than do breeding individuals of similar trunk length (Table 1). Both males and females typically reproduce annually in the San Francisco Bay area; the percentage not breeding ranged from 0 to 18% per year for the populations included in my study. Since most of these non-breeding individuals (65% compared with 17% of breeding individuals) had incompletely regenerated tails (estimated as less than 75% the length of unbroken or fully regenerated tails), autotomy, subsequent regeneration or both are interpreted as major depressants of reproductive output in these populations.

Second, maturity may be delayed as a consequence of tail autotomy. In all but one comparison, immature animals, similar to breeding individuals in trunk length and presumably age, had significantly shorter tails (Table 1) due to incomplete regeneration (25% compared with 3% of breeding individuals).

Third, field evidence suggests that tail autotomy not only inhibits reproduction but also reduces the amount of reproductive output for individuals which do reproduce.

Table 1 Comparison of relative tail length between breeding and nonbreeding salamanders of similar trunk length

	Redwood forest		Conifer woodland		Eucalyptus woodland	
	Females	Males	Females	Males	Females	Males
Not breeding, immature	— 1.8 (23)	— 5.7 (26)	— 7.7 (20)	— 1.4 (11)	—	—
Breeding	1.8 (12)	— 0.5 (14)	— 0.6 (22)	— 1.2 (19)	—	—
Probability	0.12	0.03	0.0015	0.07		
Not breeding, mature	— 9.2 (13)	— 12.8 (21)	— 23.2 (6)	— 18.3 (1)	— 26.1 (1)	— 23.8 (4)
Breeding	— 2.1 (22)	— 4.4 (25)	— 5.2 (14)	— 16.5 (3)	— 5.2 (4)	— 8.8 (4)
Probability	0.02	0.0004	0.0003	0.94	0.0001	0.135

Values are the means of the deviations (mm) of observed tail lengths from the expected unbroken tail length, calculated from the linear regression of tail length on trunk length for individuals that had unbroken or fully regenerated tails. These regressions were based on samples collected in the same habitats at different times than the samples used in this analysis. Only breeding individuals that were within 1 mm of the trunk length of nonbreeding individuals in the same collection were used. Data for each habitat are pooled from samples collected over several years. Numbers in parentheses are sample sizes. Student's *t* test was used to determine the significance of the differences; probabilities are for a two-tailed test.

In all but one case, tail length showed a significant positive partial correlation with an index of reproductive effort (the number of ovarian eggs of gravid females and the extent to which the neck muscles are enlarged in males, expressed as the ratio of neck width to head width) when trunk length is constant (Table 2).

The inhibitory influence of tail loss on reproductive output may derive simply from loss of considerable energy reserves available in the long tail or from use of remaining energy reserves, such as those in abdominal fat bodies or in the tail remnant, to regenerate the tail during the dry season instead of reproducing. Hendrickson⁴ concluded on the basis of recaptures of marked animals that regeneration occurs during both the wet and dry seasons. To test the possibility that tail regeneration during the dry season depresses reproductive output I collected 57 salamanders before the dry season (early May) and removed the whole tail from 27 of them; the rest served as controls. The

over its use to supply energy for reproduction. An explanation for this priority is that the environment has selected for longevity in *B. attenuatus* at the expense of reproduction when the two selective pressures are in conflict⁶. The environment is unpredictable to this salamander with respect to both the length of the dry season and the amount of rain during the wet season. Variability in rainfall produces variability in the amount of energy an adult can store for reproduction during the dry season. After a wet season with half the normal rainfall (1971–72), females in two habitats had 6% and 11% fewer eggs ($P=0.12$ and 0.0002 , respectively, one-tailed *t* test) than after a wetter than normal season (1972–73). Variability in the length of the dry season is thought to produce variability in mortality, particularly in juvenile mortality⁶. Averaging ten times the weight of first year juveniles, adults have a metabolic advantage in surviving prolonged periods without food, and thus survival is more certain for them than for juveniles.

Table 2 Partial correlation coefficients of trunk length and tail length with an index of reproductive effort

	Redwood forest	Conifer woodland	Eucalyptus woodland
Females			
Sample size	42	78	46
$r_{SVL, RI, TL}$	0.53 ($P < 0.001$)	0.64 ($P < 0.001$)	0.77 ($P < 0.001$)
$r_{TL, RI, SVL}$	0.36 ($P < 0.05$)	0.34 ($P < 0.01$)	0.55 ($P < 0.001$)
Males			
Sample size	28	34	
$r_{SVL, RI, TL}$	0.40 ($P < 0.05$)	0.53 ($P < 0.01$)	
$r_{TL, RI, SVL}$	0.22 ($P = 0.32$)	0.55 ($P < 0.01$)	

Both tail length and the index of reproductive effort are a linear function of trunk length. Calculations include only actively breeding individuals. SVL, trunk length; TL, tail length; RI, reproductive index (see text for explanation).

salamanders were maintained, unfed, on a natural photoperiod at 13 °C until September, at which time they were killed and examined for tail regeneration and reproductive condition. (Forty salamanders, which had been collected on a different day than the others, died shortly after the start of the experiment, probably from heat stress when collected³.)

Clearly the salamanders regenerate the tail at some expense of reproduction. All experimental animals ($n=9$) regenerated their tails at an average rate of 2.6 mm per month. All but one of them failed to become fully reproductive; all controls ($n=6$) became reproductive ($P=0.001$, Fisher's exact test).

Thus, field observations and one experiment suggest that autotomy of the tail depresses reproductive output, at least in part, because the regeneration is taking place when energy reserves are needed for reproduction. I conclude that the use of the tail for defence has functional priority

Both variability in the production of young and uncertainty in their survival are predicted to favour increased longevity at the expense of reproduction⁷.

It is not surprising that tail regeneration (and therefore defence) has priority over reproduction, for such priority is theoretically expected when selection favours longevity. It is more perplexing that the salamander does not use some other structure for either energy storage or predator defence. Although separate systems may have been 'evolutionarily available' to species, the dual use of the tail may be adaptive in that it can increase the responses of the salamander in allocating energy for reproductive and somatic functions. A specialised defence system, such as a toxin, costs the salamander a certain amount of energy that is then irrevocably tied up in the system. In contrast, by using a structure for defence that can also be used to accumulate required energy reserves, the salamander can draw on these reserves for reproduction if they are not

used in defence. When energy availability fluctuates greatly, it seems adaptive to have an occasional opportunity to expend a maximal amount of energy for reproduction.

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Coral-snake pattern recognition and stimulus generalisation by naive great kiskadees (Aves: Tyrannidae)

THE neotropical coral-snake complex with its contrasting ringed patterns includes true coral snakes, for example, the highly venomous elapid genus *Micrurus*, and many colubrids that are either less strongly venomous or non-venomous¹. Whether or not this complex involves Batesian and/or Müllerian mimicry has been widely debated²⁻⁸. Wickler¹ suggested that if mimicry depends on predator learning, true coral snakes are too deadly to be models; rather, they are mimics of the less dangerous colubrids of the complex (Mertensian mimicry). He also claimed that there was no evidence that any predator could recognise a coral snake innately¹. I have shown that Costa Rican turquoise-browed motmots (*Emomota superciliosa*) need no learning to show strong aversion to a pattern of wide yellow and narrow red rings⁹; this I have interpreted as an innate recognition of a generalised coral-snake pattern. No Costa Rican member of the coral-snake complex has wider yellow than red rings, however, so I had no proof that the motmots were not simply showing an aversion to a general aposematic pattern. I present here evidence that another avian predator needs no learning to avoid not only a pattern of wide yellow and red rings, but also the most common local *Micrurus* pattern of red, yellow and black rings.

The great kiskadee (*Pitangus sulphuratus*) occurs in open and semi-open habitats from southern United States (Texas) to south Argentina. It regularly eats small reptiles⁸, and *Micrurus* and many other members of the complex occur over much of its range. These snakes could be dangerous to kiskadees. *Micrurus* in particular may lie partly concealed in leaf litter during the day, often with its head under leaves. A kiskadee attacking the exposed portion of such a snake would be in danger of being bitten, especially if that portion did not include the head. As kiskadees lack heavy protecting scutes on their legs, such a bite could well prove fatal.

Six young kiskadees from three broods aged 12–20 d were removed in May 1976 from their nests in north-western Costa Rica and raised in the laboratory in 91×46×46-cm hardware cloth cages. Two cages each held two birds; the other two held one each. Experiments in these cages were begun when the kiskadees were old enough to catch their own prey, that is, 45 d. In each experiment a wooden dowel model 6 cm long and 1 cm in diameter was placed on the cage floor and the number and location of the birds' pecks

was recorded for 5 min. Models were painted with non-toxic tempera colours in the following five patterns: white with narrow green rings; yellow with narrow red rings; yellow with narrow red stripes; 'coral-snake' rings (wide red, narrow yellow, wide black, narrow yellow); and coral-snake stripes. Two models were made for each pattern: a 'solid' model completely covered with the pattern, and an 'end-third' model having only one end painted, the other two thirds being plain wood (Fig. 1). With the exception mentioned below, all birds received the patterns in the above order.

Each kiskadee had previously attacked solid models of plain white, green, red, yellow and black without hesitation. Of the five patterns on solid models, two were completely avoided, two readily attacked, and one attacked with great caution (Table 1). Although no kiskadee approached either the yellow and red ring or the coral-snake ring model, the responses to the two differed. Only two birds gave mild alarm calls to the former; the same two plus at least two more gave high intensity alarm calls (like those given by a captured bird) to the latter. Their response to the white and green ring model (the first pattern any bird received) shows that they did not fear ring patterns in general. Nor did they hesitate to attack the yellow and red stripe model, which has a relative proportion of yellow and red identical to that of the ring pattern. Unexpectedly, although all six birds approached the solid coral-snake stripe model, all showed great hesitation, and the four birds that finally did attack did so by darting forward with spread wings and contour feathers tightly in.

With the end-third models, the birds directed most of their pecks to the painted third of the white and green ring, the yellow and red stripe, and the coral-snake stripe models, but gave a significant response to the end farthest away from both the yellow and red ring and the coral-snake ring models (Table 1). Kiskadees thus consistently avoid the coral-snake ring and the yellow and red ring patterns, but hesitate to attack the coral-snake stripe pattern only when it covers the entire model.

This aversion to the two ring patterns seems to require no learning. Kiskadees have covered nests, and the three youngest birds barely had their eyes open when they were taken, so it is unlikely that they perceived any patterns in the dim light within their nest before their capture.

Coppinger⁹ found that naive passerines of three species gave alarm calls and active escape behaviour to a large variety of novel stimuli, the degree of rejection depending on the amount of stimulus change and the birds' previous experience. The order in which the kiskadees received the

Fig. 1 Models used in the experiments. *a*, Solid forms. *b*, End-third forms. The basic patterns were: 1, white and green (or yellow and red) rings; 2, yellow and red stripes; 3, coral snake rings, and 4, coral snake stripes. The three sections of the end-third models were recorded as p, m and u.

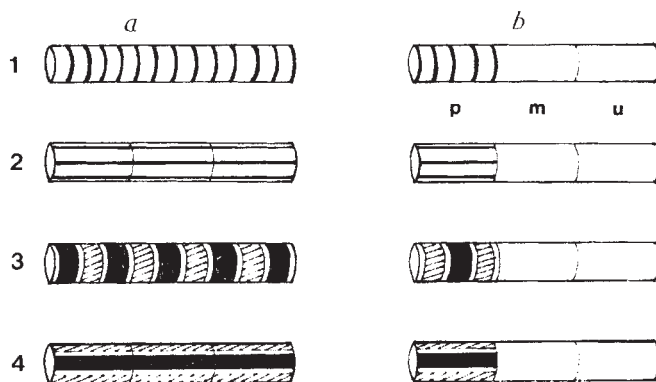


Table 1 Kiskadees' pecks to the ten models

Pattern	Pecks at solid models		Pecks at end-third models		Total pecks	χ^2 *
		p	% at each area	u		
White and green ring	59	100	—	—	51	p
Yellow and red ring	—	17.86	—	82.14	28	u
Yellow and red stripe	44	100	—	—	45	p
Coral-snake ring	—	11.11	—	88.89	9	u
Coral-snake stripe	7	100	—	—	23	p

*p indicates a response to the painted third at $P < 0.01$; u indicates a response to the unpainted end at $P < 0.05$.

models was designed to test for such novelty effects. The first pattern given was the white and green ring, which all birds attacked without hesitation: neither fear of patterns themselves nor fear of rings can thus be attributed to novelty. Because the three oldest birds had received the coral-snake ring model before the coral-snake stripe model, however, their hesitation to attack the latter might have been due to their earlier experience. The three youngest birds were therefore given the stripes before the rings. They responded in a manner similar to that of the older birds (Table 1), showing that this hesitation was not caused by previous experience.

The kiskadees' avoidance of the two ring patterns thus seems to be innate. It is unlikely that two separate innate aversions are involved; their stronger rejection of the coral-snake models, both rings and stripes, suggests that great kiskadees have an innate aversion to the coral-snake pattern, yet will generalise to avoid a pattern of wide yellow and narrow red rings.

Pattern generalisation by predators is essential for the functioning and evolution of both Batesian and Mullerian mimicry^{1,10}. If mimics evolve by microevolution, broad stimulus generalisation by local predators is necessary to give selective advantage to early divergent phenotypes just beginning to approach the model in pattern. For predators that have learned to avoid a pattern, such generalisation is well documented¹¹⁻¹². My results show that generalisation can also occur with an innate aversion. In the presence of such predators, classical Batesian or Mullerian mimics can theoretically evolve even when the model is lethal; furthermore, for Batesian mimics, the model need not be numerically more abundant than the mimic¹³. If innate recognition and generalisation of the coral snake pattern is a common phenomenon, the Mertensian mimicry hypothesis is unnecessary to explain the evolution and maintenance of the coral snake mimicry complex.

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Divergent taste responsiveness to fruit of the tree *Antidesma bunius*

GENETIC divergence of taste responsiveness was first demonstrated for phenylthiocarbamide (PTC)¹, the inability to taste PTC bitter being described as an inherited Mendelian recessive character, and ability to taste PTC bitter as dominant². This hypothesis has largely been confirmed^{3,4} although occasional incomplete penetrance of the 'taster' gene has been reported. Recently, eight people were served with a pie made from antidesma berries, and two people complained that the pie was extremely bitter and inedible. The other six people, however, found the pie pleasant tasting, enjoyably edible and sweet. This incident prompted us to survey taste responsiveness to antidesma. We found that all subjects who tasted antidesma as bitter found PTC not bitter, whereas no subject who tasted PTC as bitter found antidesma bitter.

Fresh berries from the tree *Antidesma bunius* were pressed and an aqueous extract derived. The extract was stored at 4 °C. A saturated solution of PTC (Baker) was also prepared. For the test each solution was placed into a 60-ml dark-coloured glass-dropper bottle fitted with a glass dropper.

The subjects were 170 volunteers (88 males) from the greater Washington DC area, aged 7-89 yr. The group included eight blacks, two orientals and 160 Caucasians of diverse national origin. To test taste responsiveness one drop of antidesma extract and PTC was placed successively on the tongue and the subject was requested to taste it. Each subject was also asked whether the drop tasted salty, bitter, sweet or sour and to judge the intensity of its saltiness, sweetness, sourness or bitterness on a scale from 1-100 based on previous taste experience⁵. The antidesma extract was always given first and was followed by the PTC within 5 min. Age and sex of each subject were noted as was the presence of ichthyosis. A modified form of the Ishihara colour plates⁶ was shown to each subject in a standard manner in order to identify the presence of colour blindness.

Responders to antidesma extract and to PTC were defined as subjects who described the respective solutions as bitter; non-responders were defined as those who described it either as tasteless or another taste quality. Intensity responses were calculated as the median and the mean responses of the taste responders or non-responders for each respective group.

Responders to antidesma extract comprised approximately 15% of the subjects tested, non-responders, 85% (Table 1); responders to PTC comprised approximately 68% of the group tested, non-responders, 32% (Table 1). There was no relationship in responsiveness to either antidesma or PTC with age, sex, race, national origin, colour blindness or ichthyosis. Most antidesma responders judged antidesma as bitter, the mean response being about 39% as bitter as the bitterest substance ever tasted. Most PTC responders judged PTC as about twice as bitter as the antidesma responders judged antidesma, the mean responsiveness being approximately 82%. Among the 145 non-responders to antidesma, 67 stated that it was slightly sour, 39% that it was sweet,

Table 1 Comparison of PTC and antidesma responders and non-responders

PTC	Responders	Non-responders
Subjects	115	55
Sex	57 males; 69 females	31 males; 22 females
Mean age (yr, range)	44 (9-78)	41 (7-89)
Mean response intensity (% range)	81.6 (5-100)	1.5 (0-30)
Median response intensity (%)	100	0
Median response quality (response, no.)	Bitter (115)	Tasteless (47)
Mean response frequency (%)	67.6	32.4
Antidesma		
Subjects	25	145
Sex	16 males; 9 females	71 males; 83 females
Mean age (yr, range)	43 (7-89)	42 (9-78)
Mean response intensity (% range)	39 (5-100)	24 (0-100)
Median response intensity (%)	20	12
Median response quality (response, no.)	Bitter (25)	Sour (67)
Mean response frequency (%)	14.7	85.3

29 stated that it was salty and 10 could not designate any specific taste quality.

Among the 25 responders to antidesma there were no responders to PTC and, conversely, among the 115 responders to PTC there were no antidesma responders.

Three families in which antidesma responsiveness was identified were studied. In each family the father was the index subject identified. In one family, the wife and each of the six children (three girls, three boys) were antidesma non-responders. In another family, the wife and two children (one girl, one boy) were antidesma non-responders. In the last family, the wife, paternal mother and father and three children (one girl, two boys) were studied; the paternal father and one son, an addition to the father previously identified, were antidesma responders. Among the latter two families there were no genetic markers to characterise the antidesma responders with respect to red blood cell group, type, phenotype, Rh factor or plasma Xga. One family in which both husband and wife were PTC non-responders was studied; both were antidesma non-responders and both of their children (one boy, one girl) were PTC and antidesma non-responders.

Although taste divergence for antidesma extract has not been described before, the percentage of subjects in whom taste responsiveness to PTC was found in this survey agrees closely with those of previous investigators^{7,8} as do the response characteristics of the subjects studied⁹. The active substance responsible for the taste responsiveness of the antidesma extract has not been identified although it is water soluble and heat stable. The genetics by which taste divergence to antidesma is determined is unclear, although it seems to occur among PTC non-responders comprising about half of this group. This suggests that the bitter response to antidesma among PTC non-responders bears some relationship to those factors which determine PTC taste responsiveness either on a functional or genetic level.

The divergence of taste responsiveness to antidesma raises several questions. First, antidesma fruit, unlike PTC, has been used as food by natives in South-East Asia and Florida for many years and has been eaten in pies, jams and sauces or as raw fruit. Divergent tastes to this food raises the question of the presence of other substances which may appear in the diet in which individual differences in taste perception and hence, preference, may occur. Although bitter tasting substances are not usually preferred as food or drink, preferences of the majority may exert significant influence on food practises. Thus, the number of substances to which divergent taste responses may occur and how much they may influence food and drink preference and intake are unknown. Second, these data raise the question of whether preferences for some food and drink may be determined, in part, by genetic factors. Third, individual taste responsiveness to various naturally occurring

substances may serve as useful markers to individual differences, not only to food and drink preferences, but also to drug responsiveness¹² and sex differences¹³. Lastly, divergent taste responsiveness to antidesma and PTC as contrasted with the more usual uniform bitter taste responsiveness to most alkaloids¹⁴, may be useful in the study of the psychophysical and biochemical characteristics of bitterness.

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Surface charge and asbestos toxicity

ASBESTOS is a serious occupational and environmental hazard because of its fibrogenic and carcinogenic actions on the lung. Asbestos fibres exist in various physical forms, all of which are toxic. It is not known, however, which form is the most hazardous or which fibre property is the most important for inducing the toxic effects. It is thus important to examine relationships between the structure of the fibres and their biological activity. We have investigated the relationship between the surface charge and the haemolytic activity (ability to rupture the erythrocyte membrane) of asbestos. We report here that different forms of asbestos can be activated or inactivated by surface charge alterations.

Asbestos fibres are divided into two classes, chrysotiles

and amphiboles, on the basis of their crystal structure^{1,2}. Chrysotiles are characterised by hollow cylindrical fibres formed by spiralling a double sheet of silica and brucite ($\text{Mg}(\text{OH})_2$) with the brucite on the outer surface. Amphiboles are characterised by fibres formed by stacking double silica chains back-to-back with a layer of hydrated cations in between. Owing to their dissimilar crystal structure and chemical composition, the two classes also differ in their physicochemical properties. These differences are reflected in short-term *in vitro* effects, chrysotiles being more cytotoxic to macrophages^{1,3,4} and more haemolytic^{1,5-7} than amphiboles. For long-term *in vivo* toxicity, however, there is no clear specificity in the fibrogenic and the carcinogenic activities of the two classes^{1,8-11}. This paradox of short-term and long-term asbestos toxicity has not been resolved, but factors such as surface charge¹²⁻¹⁷ and dimensions^{11,18,19} of the fibres, deposition and retention of the fibres in the lung^{19,20}, coating²¹ and dissolution²² of the fibres by lung secretions, and susceptibility of the fibres to phagocytosis^{1,8} may be significant. We have suggested that the haemolytic activity of asbestos is principally determined by the absolute magnitude of the surface charge that the fibres acquire in solution²³. For chrysotiles the surface charge is associated with structural magnesium atoms which are known to be leached from the fibre surface *in vivo*²², suggesting that progressive shifts in absolute surface charge could account for the discrepancy in short-term and long-term asbestos toxicity. We report here the effect of leaching on the surface charge and the haemolytic activity of representative samples of both classes of asbestos.

Surface charge, represented by the zeta potential²⁴, and haemolytic activity were measured for two asbestos reference samples of the International Union against Cancer (IICC) (ref. 25)—chrysotile A and the amphibole crocidolite. The fibres were leached as 0.5% (w/v) suspensions in Tyrode's (physiological buffer) solution, 0.01 M HCl or 0.1 M HCl. On each day of a leaching period, the fibres were centrifuged (3,090g, 20 min) and the leachate was siphoned and

Fig. 1 Zeta potential in absolute units (Z) at pH 7.4 for asbestos fibres leached with Tyrode's solution. ○, UICC chrysotile A; ●, UICC crocidolite.

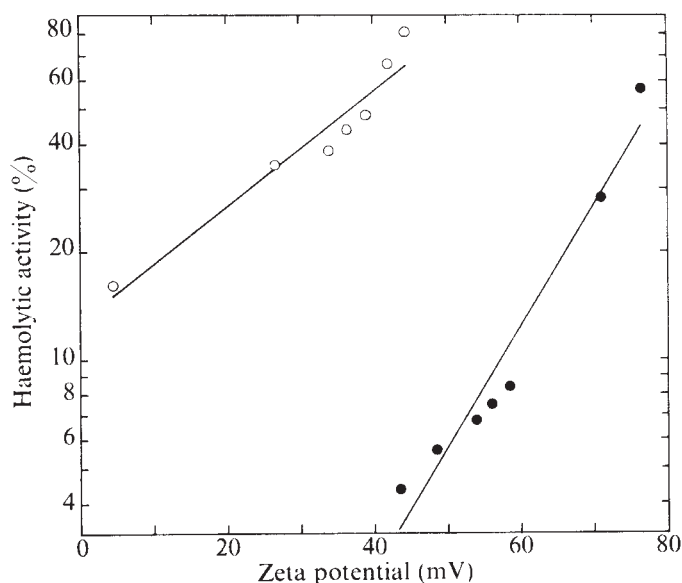
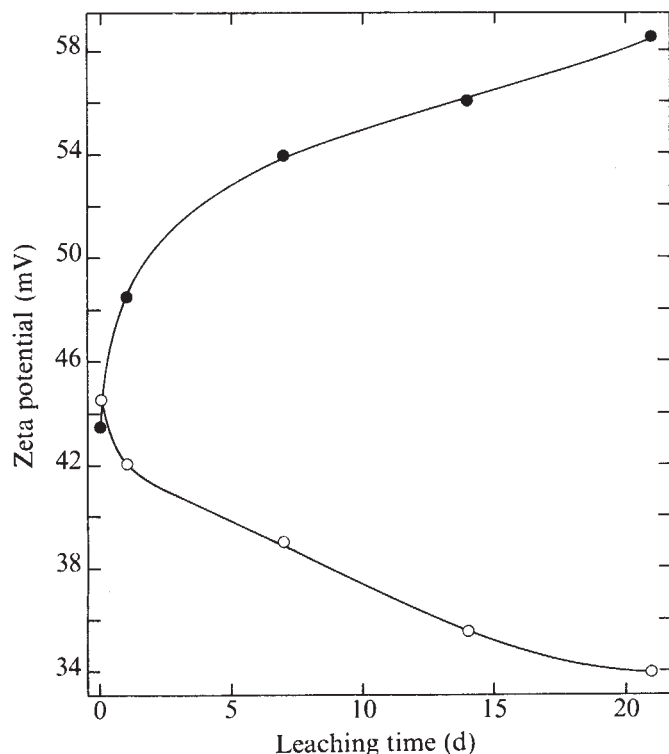


Fig. 2 Correlation between zeta potential in absolute units (Z) and haemolytic activity for leached asbestos fibres. ○, UICC chrysotile A ($r = 0.96$); ●, UICC crocidolite ($r = 0.97$).

replaced. The HCl leachings were for 1 d. The electrophoretic mobilities of fibres incubated for 2 h as 0.01% (w/v) distilled-water suspensions were measured using a microelectrophoresis instrument (Zeta-Meter). From these data, zeta potentials were approximated using the Helmholtz-Smoluchowski equation²⁴ which is generally considered to apply for cylindrical particles placed axially to the electric field. For the haemolysis tests, fibre samples of equal surface area (20 mg crocidolite, 7.7 mg chrysotile A) were incubated for 1 h in 2 ml of Tyrode's solution; 2 ml of a 1.5% (v/v) Tyrode's-solution suspension (pH 7.4) of washed sheep erythrocytes was then added. The mixtures were inverted twice every 10 min for 1 h at 23 °C. The optical densities were measured at 541 nm after centrifugation (495g, 20 min).

Zeta potentials at pH 7.4 for the two types of unleached asbestos were similar in absolute magnitude, but opposite in polarity: +44.5 mV for chrysotile A and -43.5 mV for crocidolite. On leaching, the absolute magnitude of the zeta potential (Z) decreased for chrysotile A, and increased for crocidolite. The rate of change in Z at pH 7.4 for chrysotile A and crocidolite leached with Tyrode's solution is shown in Fig. 1. After a sharp change in Z during the first 5 d, the variation became nearly linear and at 21 d the Z values for chrysotile A and crocidolite were 34.0 and 58.5 mV, respectively. For both chrysotile A and crocidolite, there was a proportional shift in haemolytic activity as Z changed on leaching. Data for fibres leached with HCl and Tyrode's solution (Fig. 2) show the highly significant ($P < 0.01$) correlation between Z and haemolytic activity for the two types of asbestos.

These results confirm the importance of surface charge to haemolytic activity and show that fibre dissolution can markedly alter the magnitude of surface charge and hence, activate or inactivate the fibres. The positively charged chrysotile was initially more active than the negatively charged amphibole. Leaching of the fibres caused the absolute magnitude of surface charge and the corresponding haemolytic activity of the chrysotile to decrease progressively and that of the amphibole to increase progressively. With continuous leaching *in vivo*, surface charge could be altered so that the biological activities of chrysotiles and amphiboles converged. This phenomenon could explain why the two classes of asbestos fibres, although different in

their short-term *in vitro* effects, are not clearly different in their long-term toxicities.

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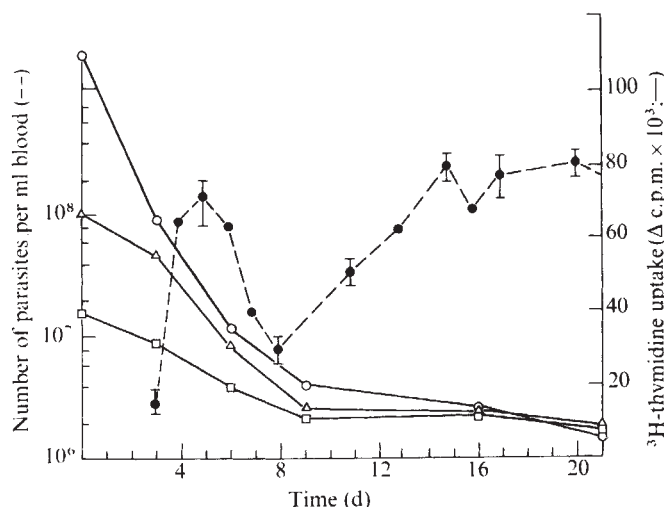


Fig. 1 Mean parasitaemia (●) $\pm$ s.e. and mean DNA synthetic responses to con A (○), PHA (□) and LPS (△) of spleen cells of CBA/CaJ mice infected with S 42 *T. brucei*. Standard errors were $<5\%$ in all cases. Mean parasitaemia was estimated from haemocytometer counts made on the peripheral blood of six mice. DNA synthesis was measured by Williams⁹ method. Viable spleen cells (5×10^5) were cultured in 0.1 ml RPMI 1640 containing 5% foetal calf serum (Gibco) with optimal concentrations of mitogens. Con A (Calbiochem) was used at a final concentration of $2 \mu\text{g ml}^{-1}$, PHA (Difco) at $0.31 \mu\text{l ml}^{-1}$ and LPS (LPS 055:B5, Difco) at $50 \mu\text{g ml}^{-1}$. All cultures were set up in flat-bottomed tissue culture plates (Linbro) and were incubated for 72 h at 37°C in a humidified atmosphere of $5\% \text{CO}_2$ in air. Cultures were pulsed with $1 \mu\text{Ci } ^3\text{H-thymidine}$ (New England Nuclear, specific activity 5 Ci mmol^{-1}) 16 h before collection. At each stage of infection, spleen cells were pooled from groups of three mice and cultures were set up in triplicate. Results are expressed as mean c.p.m. in stimulated cultures minus mean c.p.m. in unstimulated cultures (Δ c.p.m.).

Suppressor cells in experimental trypanosomiasis

HUMAN and experimental forms of African trypanosomiasis are characterised by a profound suppression of immunological responsiveness. Suppression of both humoral and cell-mediated responses has been observed. *Trypanosoma gambiense* infections in man reduce antibody responses to *Salmonella typhi* (H antigen) and skin reactivity to purified protein derivative, *Candida* and dinitrochlorobenzene¹. *T. brucei* infections in mice cause marked suppression of antibody² and splenic plaque-forming cell^{3,4} responses to sheep red blood cells (SRBC), and in rabbits suppress the development of experimental allergic neuritis⁵. The mechanism underlying this inhibition of immunocompetent cell function has not been elucidated. We have examined the DNA synthetic responses of spleen cells from *T. brucei*-infected mice to concanavalin A (con A) and phytohaemagglutinin (PHA) (both T-cell mitogens), *Escherichia coli* lipopolysaccharide (LPS, a B-cell mitogen) and allogeneic cells, and the ability of these cells to influence the responses of normal cells. We present evidence suggesting that suppressor cells^{6,7} are involved in the immunological hyporesponsiveness observed in this disease.

Infection of CBA/CaJ mice with S 42 strain *T. brucei* produced a progressive and uniformly fatal infection (Fig. 1). After the inoculation of 10^4 trypanosomes (all infections were initiated by the intraperitoneal inoculation of 10^4 trypanosomes derived from stablate⁸ material) parasites were detected in the peripheral blood by day 3 and increased rapidly until day 6. Parasite levels then regressed until day 8 or 9, after which they increased steadily and proved fatal in 30-35 d.

DNA synthetic responses of spleen cells from infected mice to con A, PHA and LPS were suppressed as early as day 3 of infection (Fig. 1) and the suppression increased as the infection progressed. The degree of suppression

observed did not always seem to be directly proportional to blood parasitaemia. The ability of spleen cells from infected CBA/CaJ mice to respond to allogeneic (BALB/c) spleen cells in a mixed lymphocyte reaction was also significantly inhibited (data not shown).

As recent work^{10,11} has implicated suppressor cells with adherent properties in a comparable situation—the reduced immunological reactivity obtained in animals treated systemically with large doses of protein antigen—we examined the possibility that the nonspecific suppression in *T. brucei*-infected mice was mediated by an analogous mechanism.

Initially, we examined the effect of adding various number (6×10^4 , 1.25×10^5 , 2.5×10^5 , 5×10^5 and 7.5×10^5) of spleen cells from infected mice (at day 12 of infection) or normal mice to cultures of normal spleen cells (5×10^5) stimulated with con A, PHA, or LPS. Spleen cells from infected mice caused consistent, dose-dependent, suppression of the mitogen-induced DNA synthetic responses of the normal cells. Addition of normal spleen cells caused no significant suppression except at the highest cell concentration and this was probably due to 'crowding' effects. Spleen cells from infected mice also caused significant suppression of one-way mixed lymphocyte reactions between normal CBA/CaJ and mitomycin C-treated BALB/c spleen cells. The degree to which the DNA synthetic response of normal cells to con A, PHA, LPS and allogeneic cells was suppressed at a $1(2.5 \times 10^5):2(5 \times 10^5)$ ratio of infected to normal cells is shown in Fig. 2. The ability of 2.5×10^5 spleen cells from infected CBA/CaJ mice to suppress the responses of 5×10^5 normal spleen cells increased as the infection progressed. We found that the con A-induced DNA synthetic response was reduced to 50% of the normal response by day 6 cells, to 19% by day 12 cells and to 11% by day 20 cells.

We next examined the effect of depleting *T. brucei* spleen

cells of glass-wool-adherent cells. DNA synthetic responses of whole and nonadherent spleen cells from normal and infected mice to con A, PHA and LPS are shown in Fig. 3. Depletion of glass-wool-adherent cells caused a more substantial increase in the response of *T. brucei* spleen cells than of normal cells to PHA and LPS; the response to con A was improved, but to a lesser extent by this procedure. We have also found that glass-wool-adherent cells from infected mice consistently suppressed mitogen-induced responses of normal spleen cells 25–30% more than the unfractionated population; peritoneal macrophages from infected mice had no effect. Hence the suppressor cell seemed to have adherent properties but was distinct from the peritoneal macrophage.

It seemed possible, however, that some of the suppression observed was a direct inhibitory effect of trypanosomes present in spleen cell suspensions from infected mice—at day 12 of infection, the spleen cell to trypanosome ratio was approximately 30:1. To investigate this possibility we separated trypanosomes from the peripheral blood of infected mice¹³ and added trypanosomes (4×10^3 – 2×10^5) to cultures of normal spleen cells (5×10^5) which were then stimulated with con A or LPS; these numbers of trypanosomes caused no suppression, indicating that the suppression in our cell mixture experiments was mediated primarily by a cell. This was substantiated by the observation that glass-wool-adherent cells, although more suppressive, were contaminated by 1/10 the number of trypanosomes present in the whole spleen cell population. In more recent experiments we have observed that very large numbers of trypanosomes (6×10^5 or more) suppress mitogen-induced responses of normal cells. The possibility that this suppression is also due to the induction of suppressor cell activity cannot be excluded.

Although the cell mixture and glass wool fractionation experiments clearly implicate a splenic cell in the suppression, it is possible that the presence of some trypanosome antigen in culture may augment the suppression observed. Gershon, Gery and Waksman¹⁰ found that the reduced responsiveness to PHA *in vitro* of spleen cells from animals immunised with SRBC, bovine γ -globulin and bovine serum albumin was further suppressed by the addition of the immunising antigen to the cultures.

To characterise further the cell responsible for suppression in this system, we compared the growth of the parasite in normal and congenitally athymic (nude)

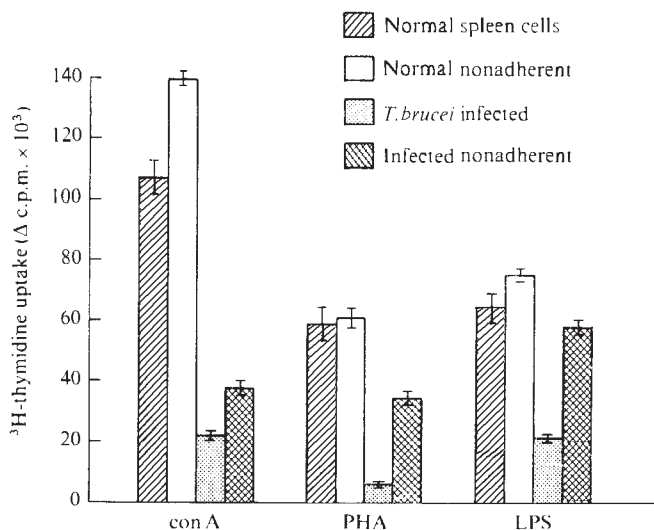


Fig. 3 DNA synthetic responses to con A, PHA and LPS (± 1 s.e.) of whole and nonadherent spleen cells from normal and *T. brucei*-infected CBA/CaJ mice. Spleen cells were pooled from four normal and four infected (day 7 of infection) mice. Adherent cells were removed on glass wool columns using the method of Folch, Yoshinaga and Waksman¹².

BALB/c mice and tested the spleen cells of these mice for suppressor activity. Parasitaemias were generally lower in nude mice than in normal controls (Fig. 4a). Although spleen cells from infected BALB/c mice caused significant suppression of mitogen responses, spleen cells from infected nude mice at the same stage of infection (day 11) failed to suppress the mitogen-induced DNA synthetic responses of normal cell (Fig. 4b), indicating that the suppressor in the spleens of *T. brucei*-infected mice was either a T cell or was dependent on T-cell influence for its generation. Treatment of spleen cells from infected mice with an anti-Thy-1 antiserum and complement, however failed to remove their suppressive effect. There

Fig. 4 Mean parasitaemias (± 1 s.e.) in normal (●) and nude (○) BALB/c mice infected with S 42 *T. brucei* (a) and the effect of spleen cells from these mice on con A, PHA and LPS-induced DNA synthetic responses of normal BALB/c spleen cells (b).

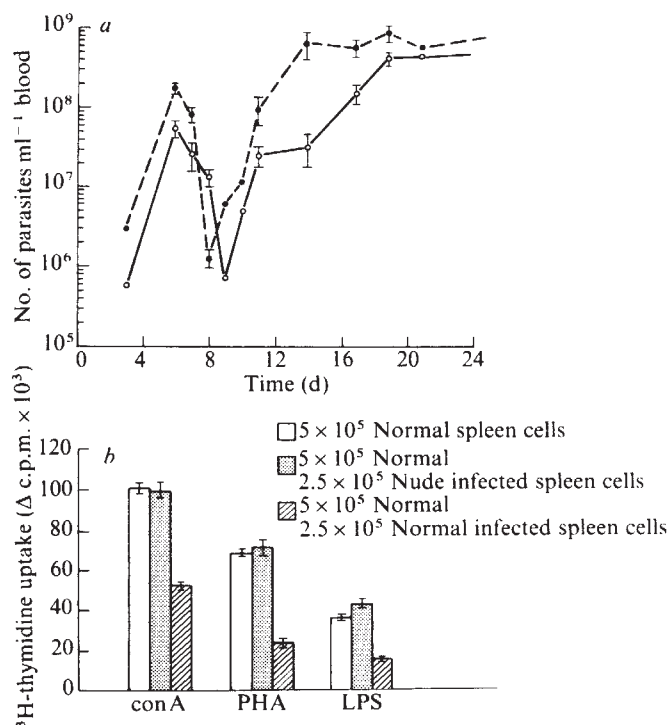
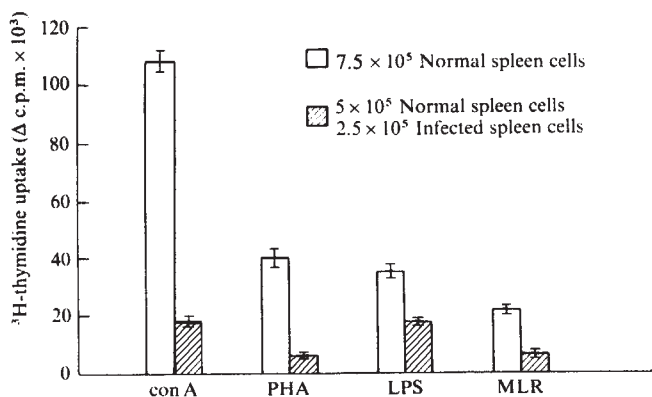


Fig. 2 Effect of spleen cells from *T. brucei*-infected CBA/CaJ mice on con A, PHA, LPS and allogeneic-cell-induced DNA synthetic responses ± 1 s.e. of normal spleen cells. Mixed lymphocyte reactions (MLR) were initiated using 2.5×10^5 CBA/CaJ spleen cells and 2.5×10^5 mitomycin C-treated BALB/c spleen cells. Spleen cells (10^7 per ml) were treated with mitomycin C ($30 \mu\text{g ml}^{-1}$, Sigma) at 37 °C for 30 min. Culture conditions were as described previously. Cultures were pulsed with 1 μCi ³H-thymidine 16 h before collection.



have been reports of suppressor T-cells which seem to express only small amounts of Thy-1 on their surface and are therefore difficult to lyse with anti-Thy-1 antisera¹⁴. Hence in spite of its insensitivity to the concentration of anti-Thy-1 antiserum which we used we cannot exclude the possibility that the suppressor in our system is a T cell.

We have also observed that unfractionated or T-cell-enriched (on nylon wool columns) spleen cells from infected mice cause marked suppression of the plaque-forming colony response of normal spleen cells to SRBC *in vitro* (unpublished results of D. D. Eardley and A.N.J.), and that the adoptive transfer of spleen cells from *T.brucei*-infected mice to normal recipients results in infections which are enhanced compared with infection in controls receiving normal spleen cells and the same number of parasites.

Although these experiments suggest that thymus-dependent suppressor cells are involved in the nonspecific suppression of immunity, it is possible that specific suppressor cells are also generated in these infections. We are attempting to analyse in greater detail the mechanism by which the nonspecific suppressor cells act and their role in the pathogenesis of trypanosomiasis.

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Immunologic studies in contacts of osteosarcoma in humans and animals

AGENT(S) which could cause unrecognised or subclinical infections in otherwise healthy human household contacts of cancer patients may be detected by epidemiologic studies. Support for this hypothesis is provided by the reports of an increase in tumour specific humoral antibodies, as well as tumour specific cell mediated immunity¹⁻⁴ in the household contacts of patients with osteosarcoma, adenocarcinoma of the breast and other malignancies. Clear evidence for the

validity of this conclusion has not been obtained. To test this hypothesis, we studied antibodies (both humoral and cellular) in the household contacts of a patient with osteosarcoma. In addition, in an animal model for human osteosarcoma, we characterised the sera and lymphocytes of hamster mothers (in close contact with their offspring) before and after induction of the tumour in their immunosuppressed offspring.

The humoral antibodies were tested by the indirect immunofluorescence test against tumour tissue imprints obtained from a patient (M.Mc.) with osteosarcoma of the femur. The membrane immunofluorescence test was performed with TE-85 cells (from an unrelated patient with osteosarcoma) maintained in tissue culture. Sera from five of the six household contacts of the patient (M.Mc.) showed positive reactions for humoral antibodies against both osteosarcoma imprints and TE-85 cells. Similar positive reactions were observed with the sera from four other (unrelated) patients with osteosarcoma and the patient's (M.Mc.) own serum for the presence of humoral antibodies. Only the serum of one of nine normal individuals showed positive immunofluorescence against both the tumour imprints and cells.

In the animal experiments, sera from mothers of tumour-bearing hamsters were subjected to the immunofluorescence test before the injection of TE-85-M-MSV cells into anti-lymphocyte serum (ALS) treated newborn hamsters. The sera from the hamster mothers were again tested on 5 and 20 d after inoculation of TE-85-M-MSV cells into the offspring (the tumours developed in the offspring 10-14 d after inoculation). Sera from eight of the 10 hamster mothers were positive for antibodies (against tumour imprints and TE-85 cells) on d 20 after the inoculation of the cells into the newborn hamsters. The sera from the same hamster mothers showed a negative reaction for antibodies on the day of the injection of cells and on d 5 after injection. None of the sera from the hamster mothers in the control group showed positive immunofluorescence.

Tumour specific antibodies were demonstrable against osteosarcoma imprints (by immunofluorescence) in the sera of 83% of the tumour-bearing newborn hamsters. 87% of the sera from these animals reacted positively against TE-85 cells. In the control group of newborn hamsters, only 4% of the animals had sera which reacted positively against osteosarcoma imprints and TE-85 cells (Table 1).

For the cell mediated immunity, the lymphocyte micro-cytotoxicity test was performed as described by Singh *et al.*⁵. The TE-85 cells maintained in tissue culture were used as target cells. Table 2 shows the percentage of inhibition of the target cells by the lymphocytes from the household contacts of the patient (M.Mc.), the patients with osteosarcoma and normal individuals. Significant inhibition by the lymphocytes was observed in five of the six same household contacts, all of the patients with osteosarcoma and one of the nine normal individuals.

In the animal model, the same eight hamster mothers had lymphocytes that exhibited significant cytotoxicity against TE-85 cells at d 20. The lymphocytes from the same mothers exhibited minimal cytotoxicity before the inoculation of cells into newborn hamsters and on d 5 after the cell

Table 1 Tumour-specific antibodies in tumour-bearing newborn hamsters

	No. of hamsters	Osteosarcoma imprints		TE-85 cells	
		Positive sera* per hamster test	%	Positive sera* per hamster test	%
Experimental group	72	60/72	83	63/72	87
Control group	23	1/23	4	1/23	4

For osteosarcoma imprints a standard indirect immunofluorescence test was performed against fixed sections. For membrane immunofluorescent 3×10^5 cells ml⁻¹ were suspended in the test serum 1:10 dilution and incubated at 4 °C for 30 min. Fluorescent conjugated goat anti-hamster γ -globulin 1:10 dilution were used.

*Sera were drawn on d 20 after the injection of TE-85-M-MSV cells. Controls were normal newborn hamsters (no exposure to TE-85-M-MSV cells).

Table 2 Cytotoxicity of lymphocytes from osteosarcoma patients and their contacts

Experiment no.	Source of lymphocytes	No. of target cells per well 48 h after exposure to:		% Inhibition of target cells
		Medium only	Medium plus lymphocytes	
1	RL (patient)	499.4 ± 21.6	189.8 ± 15.3	62.0 ± 4.99*
2	US (patient)	715.0 ± 28.6	375.4 ± 21.7	55.9 ± 3.23*
	RO (patient)		208.8 ± 17.6	70.8 ± 5.96*
3	LM (patient)	610.0 ± 28.1	128.1 ± 09.5	79.0 ± 5.85*
4	MMC (patient)	842.3 ± 12.0	433.3 ± 0.91	48.5 ± 1.02*
5	JMC (HHC)		202.6 ± 11.0	73.1 ± 3.99*
	CMC (HHC)	755.0 ± 06.4	136.0 ± 05.8	81.9 ± 3.54*
	KMC (HHC)		79.3 ± 20.9	89.4 ± 23.6*
	BMC (HHC)		183.3 ± 05.4	75.7 ± 2.24*
6	JMC (HHC)	825.3 ± 11.5	590.6 ± 07.4	28.4 ± 0.35*
	TMC (HHC)		860.6 ± 08.9	2.2 ± 0.02
7	KT (N)	568.0 ± 25.4	521.0 ± 16.7	8.2 ± 0.26
8	IS (N)	502.6 ± 26.7	451.9 ± 22.9	10.1 ± 0.51
9	JC (N)	439.4 ± 21.6	457.5 ± 19.8	8.5 ± 0.36
10	GS (N)	451.0 ± 21.8	390.6 ± 23.2	13.2 ± 0.78
11	NK (N)	610.0 ± 28.1	353.8 ± 25.6	42.1 ± 3.05*
12	DR (N)	500.2 ± 13.9	455.0 ± 11.8	9.6 ± 0.24
13	CD (N)	402.1 ± 25.6	368.4 ± 21.8	8.4 ± 0.48
14	MC (N)	842.3 ± 12.0	811.0 ± 14.9	3.6 ± 0.06
15	AB (N)	451.1 ± 21.8	462.0 ± 21.1	7.6 ± 0.34

Subjects were household contacts of patient MMC (HHC), normal individuals unrelated to patient MMC (N), and a normal control who had worked with osteosarcoma cells in the laboratory (NK). Lymphocytes from patients and normal controls were used as effector cells and TE-85 cells were the target cells for the microcytotoxicity test.

*Statistically significant, $P < 0.05$.

†Mean ± s.e.m.

injection. The lymphocytes from control animals showed no significant cytotoxic effect against TE-85 cells.

The results of the lymphocyte microcytotoxicity test, on tumour-bearing hamsters on d 20 (Table 3), showed that 75% of the animals showed significant cytotoxic effect against the target cells. In the control group, only 4% of the animals showed a significant reaction.

Hellström and Hellström³ reported cellular immunity directed against neuroblastoma in the mothers of children with that tumour. Byers *et al.*⁴ demonstrated that household contacts of osteosarcoma patients and patients with adenocarcinoma of the breast had specific immunity against the tumour type with which there had been contact. Furthermore, the range of cytotoxicity values produced by lymphocytes from the household contacts was significantly higher than that of the normal population. In the present investigation, all the osteosarcoma patients undergoing the lymphocyte microcytotoxicity test had higher cell mediated immunity against TE-85 cells than normal individuals. In the animal study, 80% of mothers of the tumour-bearing hamsters developed higher cell mediated immunity than the mothers in the control groups.

Morton and Mälgren⁵ interpreted the transformation of embryo fibroblasts (in tissue culture) as evidence for a transmissible agent (either horizontal or vertical) in

patients with the tumours. An electron microscopic finding of occasional particles resembling murine leukaemia virus (in some biopsy specimens and cells of tissue cultures derived from osteosarcoma) were considered to indicate the presence of a viral antigen or virus-induced tumour antigen in the tested tumour.

In our animal model there was an 80% incidence of humoral antibodies against osteosarcoma imprints and the TE-85 cells in the sera of mothers of tumour-bearing newborn hamsters. This was demonstrated only after the tumours developed and not before tumour development or during tumour formation. The same was true of the cell mediated immunity. The production of antibodies against osteosarcoma in the animal model suggests a change in the immune mechanisms of the hamster mothers in close contact with tumour-bearing animals, and indicates that such a change was related to the development of osteosarcoma in the animal. Our results support the hypothesis that an agent is responsible for transmission of horizontal immunity to close contacts of patients with spontaneously arising osteosarcoma, and suggest that an infectious agent may be an aetiological factor in human osteosarcoma.

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Table 3 Lymphocyte cytotoxicity in mothers of newborn hamsters 20 d after injection of TE-85-M-MSV cells in newborns

Source of lymphocytes, cage no.	% Target cell inhibition
34	29.09 ± 4.24
16	63.73 ± 11.52
33	23.00 ± 9.86
29	34.03 ± 8.44
1	33.31 ± 9.67
2	46.41 ± 7.71
4	30.69 ± 8.83
6	60.16 ± 12.62

Lymphocytes from hamster mothers at d 20 were used as effector cells against target cells (TE-85) in a ratio of 1:25 for the microcytotoxicity test. Samples for controls were taken from mothers of newborn hamsters which had been injected with saline instead of TE-85-M-MSV cells and showed no significant inhibition.

$P < 0.05$, statistically significant.

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Requirement for magnesium influx in activation of alveolar macrophages mediated by ionophore A23187

NORMAL antibacterial activity in the lung is largely dependent on alveolar macrophage function¹. Products of antigen-stimulated, sensitised lymphocytes can activate alveolar macrophages, which then exhibit greater adherence, phagocytosis and nonspecific bactericidal capacity than normal². Although the molecular basis of this activation is unknown, influx or intracellular redistribution of divalent cations (Ca^{2+} and Mg^{2+}) has been implicated in other cell types^{3,10}. We have therefore examined the effect on alveolar macrophage function of exposure to the ionophore A23187, which selectively binds divalent cations at neutral pH and passively transfers them across biological membranes^{11,12}.

Monolayers of normal rabbit alveolar macrophages were exposed for 1 h to tissue culture medium containing $5 \mu\text{M}$ A23187 or to normal medium alone. Cells were then washed and reincubated in fresh medium without ionophore for a further 18–72 h. Macrophage function (adherence to culture dishes, bactericidal activity and acid phosphatase content) was then assayed^{2,14}.

Macrophages studied 18–24 h after exposure to ionophore were similar in their properties to control macrophages, but cells left in normal medium for 40–72 h after 1 h of

contact with ionophore, showed evidence of activation (Table 1). They increased in cell agglutinability or clumping (Fig. 1), adherence to tissue culture dishes and bactericidal activity against *Listeria monocytogenes* compared with normal cells (Table 1). The lysosomal hydrolytic enzyme, acid phosphatase, was also increased in the cells stimulated by ionophore.

We considered the possibility that A23187, a *Streptomyces* antibiotic¹¹, contributes to the intracellular killing of ingested *L. monocytogenes*. This was thought unlikely

Table 1 Function of alveolar macrophages after exposure to control or A23187-containing media

Experimental group	% Cell adherence*	% Acid phosphatase content†	% Intracellular bacterial survival (2 h)‡
Control	—	—	$73.1 \pm 8.4\%$ (23)
A23187	$209.5 \pm 30.8\%$ (15)	119.6 ± 3.2 (52)	$26.2 \pm 3.1\%$ (15)

*Calculated as

$$\frac{\text{no. of macrophages on dishes exposed to A23187}}{\text{no. of macrophages on control dishes}} \times 100$$

†Calculated as

$$\frac{\mu\text{mol product/mg cell protein-macrophages exposed to A23187}}{\mu\text{mol product/mg cell protein-control macrophages}} \times 100$$

‡Calculated as

$$\frac{\text{viable intracellular bacteria at 2 h}}{\text{viable intracellular bacteria at 0 time}} \times 100$$

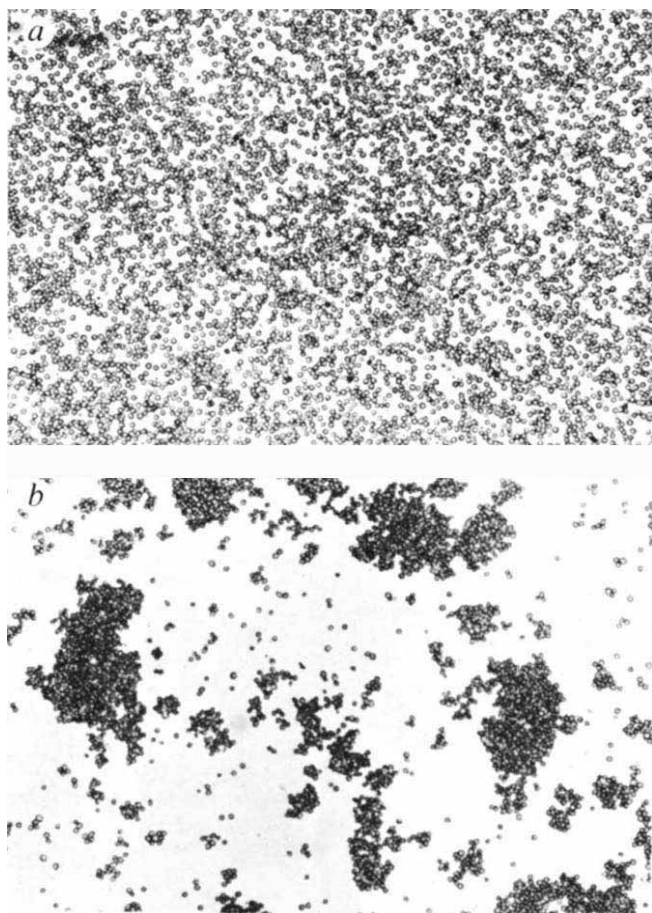
§Mean + s.e.m. Number of experiments in parentheses.

*Significantly different from control ($P < 0.005$).

†Significantly different from control ($P < 0.001$).

**Significantly different from control ($P < 0.001$).

Fig. 1 Cellular aggregation or agglutinability was perhaps the most striking manifestation of ionophore exposure. It is illustrated by photomicrographs of macrophage monolayers exposed for 1 h to A23187-containing or control media, followed by washing and reincubation in fresh medium without ionophore for 48 h. *a*, The control is characterised by a uniform layer of macrophages; *b*, marked cellular agglutination and clumping is seen after exposure to A23187 ($\times 23.5$).



Cells collected by lavage from the lower respiratory tract of male New Zealand rabbits were washed and then diluted in tissue culture medium 199 containing 15% normal heat-inactivated rabbit serum (TC 199-NRS), placed in tissue culture dishes and incubated for 4 h at 37°C in 95% air–5% CO_2 (refs 2, 13). Nonadherent cells were then removed, and the macrophages were exposed to $5 \mu\text{M}$ A23187 in TC 199-NRS or to normal medium alone for 1 h. Monolayers were washed and reincubated in fresh TC 199-NRS without ionophore for 40–72 h before studies of macrophage function. At the conclusion of this incubation, determinations of adherence to tissue culture dishes and bactericidal activity against *Listeria monocytogenes* were performed as previously described². For the acid phosphatase assay, macrophages were lysed in 0.1% Triton X-100 (ref. 14). *p*-Nitrophenyl phosphate sodium served as the substrate.

because macrophage bactericidal activity was normal at 18–24 h but had increased at 40–72 h after exposure to ionophore. Direct testing revealed that A23187 at concentrations up to $5 \mu\text{M}$ ($2.6 \mu\text{g ml}^{-1}$) in medium TC 199 with normal rabbit serum (NRS) failed to kill *L. monocytogenes* during a 2-h incubation (the time used in macrophage bactericidal assays).

To investigate the need for specific divalent cations in ionophore-mediated activation macrophages were exposed to A23187 in TC 199-NRS or in salt solutions with various concentrations of Ca^{2+} and Mg^{2+} (Table 2). Ionophore in the presence of physiological concentrations of Ca^{2+} and Mg^{2+} (tissue culture medium and balanced salt solution), or physiological Mg^{2+} with markedly decreased Ca^{2+} (10^{-5} M), stimulated acid phosphatase activity. In contrast, exposure of cells to A23187 in a salt solution containing physiological Ca^{2+} and decreased Mg^{2+} (10^{-5} M) produced no increase in enzyme activity. The addition of 10 mM MgEGTA to A23187-containing TC 199-NRS, providing a physiological concentration of Mg^{2+} ($3 \times 10^{-3} \text{ M}$) and a marked reduction in Ca^{2+} content (10^{-10} M)¹⁵, abolished the activating effect of the ionophore.

The cell-mediated immune response produces marked changes (activation) in systemic macrophages. Compared with normal macrophages, activated cells are larger, morphologically more complex, and show greater adherence, ingestion, nonspecific bactericidal activity, oxygen uptake,

Table 2 Acid phosphatase content of alveolar macrophages after exposure to A23187-containing solutions

Experimental group	Divalent cations (molar concentration)		% Acid phosphatase content*
	Mg ²⁺	Ca ²⁺	
TC 199-NRS Control A23187	1 × 10 ⁻³	1.8 × 10 ⁻³	100 119.6 ± 3.2†‡ (52)
Balanced salt solution Control A23187	1 × 10 ⁻³	1.8 × 10 ⁻³	100 110.7 ± 3.7§ (17)
Low Ca ²⁺ salt solution Control A23187	1 × 10 ⁻³	1 × 10 ⁻⁵	100 114.5 ± 6.9¶ (22)
Low Mg ²⁺ salt solution Control A23187	1 × 10 ⁻⁵	1.8 × 10 ⁻³	100 95.2 ± 2.7 (12)

The experimental procedure was as described in Table 1, except that in the experiments indicated the exposure of macrophages to ionophore took place in specified salt solutions rather than in TC 199-NRS. These salt solutions were Earle's or Hanks' balanced salt solution (BSS) or 'calcium- and magnesium-free' BSS, to which Ca²⁺ or Mg²⁺ was added in the indicated concentration. After brief exposure to A23187-containing or control medium, cells were washed and reincubated in fresh culture medium (with normal Ca²⁺ and Mg²⁺ and without ionophore) for 48–72 h before assay.

*Calculated as $\frac{\mu\text{mol product per mg cell protein-macrophages exposed to A23187}}{\mu\text{mol product per mg cell protein-control macrophages}} \times 100$

†Mean ± s.e.m. Number of experiments in parentheses.

‡Significantly different from control ($P < 0.001$).

§Significantly different from control ($P < 0.025$).

¶Significantly different from control ($P < 0.05$).

glucose oxidation and content of hydrolytic enzyme¹⁶. Acquired resistance to systemic infection with many intracellular organisms depends on these changes. Our demonstration that lymphokines activate alveolar macrophages has implied that this protective mechanism is also intact in the lung². Certain common features of activation in other cell types suggest a potential mechanism for the lymphokine-mediated process in macrophages. Influx or intracellular redistribution of divalent cations has been implicated as the initiating event in activation of various cells, including lymphocytes, fibroblasts and sea urchin eggs^{3–10}. Much of the evidence for the importance of divalent cations (especially that ascribed to Ca²⁺) in cellular activation was obtained using A23187 (refs 3, 5–7).

There are at least three possible mechanisms by which A23187 and divalent cations might initiate cell activation. First, a requirement for Ca²⁺ may be related to induced increases in intracellular cyclic GMP (refs 10, 17, 18). Increases in cyclic GMP concentration (and/or decreases in the ratio of cyclic AMP : cyclic GMP) are associated with proliferation or activation in lymphocytes and fibroblasts^{19–23}. Second, the postulated importance of Mg²⁺ in the stimulation of cell proliferation (chick embryo fibroblasts) could be related to the requirement of this cation for transphosphorylation reactions. Thus increasing the concentration of intracellular free Mg²⁺ may activate cellular metabolism and growth^{9,24}. Third, A23187 may initiate activation by producing a charge-neutral exchange of 2 H⁺ for each divalent cation transported across the plasma cell membrane^{11,12,25}. This H⁺ efflux (exchange of 2 H⁺ for each Ca²⁺ or Mg²⁺) may lead to an increase in intracellular cytoplasmic pH; such a change in intracellular pH has been reported to initiate activation of sea urchin eggs²⁶.

In view of these possibilities we examined the requirements for initiation of alveolar macrophage activation. We found that to activate normal alveolar macrophages A23187 needs both near-physiological concentrations of extracellular Mg²⁺ and very low concentrations (between 10⁻³ and 10⁻⁵ M) of Ca²⁺. Thus Ca²⁺ influx alone is not the initiating event for A23187-induced macrophage activation. Our results indicate that Mg²⁺ is the major extracellular divalent cation required at the onset of activation. These results apparently contrast with reports that Ca²⁺ influx is the initial event in ionophore-mediated activation of other

cells^{3,5–7}. It is also true, however, that in some of these studies Ca²⁺ influx was merely assumed to be the basis of the A23187 effect, and other possibilities were not excluded (such as a role for Mg²⁺). In any case the need for Mg²⁺ in the activation of alveolar macrophages is clear, although the mechanism involved is not.

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Interrelationship of cyclic nucleotides and anaphylactic reactions

THE mode of action of several anti-allergic drugs, which are structurally diverse, has been studied by comparing their inhibition of cyclic nucleotide phosphodiesterase with inhibition of anaphylaxis. The cyclic nucleotide phosphodiesterase activities were measured in microsomal preparations of both human and sheep lung, and the anaphylaxis was observed *in vitro* in passively sensitised human lung and in rats *in vivo* (passive cutaneous anaphylaxis (PCA)). A significant correlation has been found between the inhibition of anaphylaxis and the ratio of the inhibition of hydrolysis of cyclic AMP to the inhibition of hydrolysis of cyclic GMP. A less significant correlation was also found between inhibition of rat PCA and inhibition of hydrolysis of cyclic GMP.

The studies of Austen *et al.*¹⁻³ have shown that inhibition of anaphylactic release of histamine from human lung tissue can be associated with raised tissue levels of cyclic AMP. Lichtenstein and Margolis⁴ have shown that methylxanthines inhibit the antigen-induced release of histamine from human leukocytes and it has been assumed that this is due to their ability to inhibit cyclic AMP hydrolysis. A relationship has been suggested⁶⁻⁸ between the activity of anti-allergic compounds and their ability to inhibit cyclic 3',5'-nucleotide phosphodiesterases. Austen *et al.*⁴

have also suggested that, in contrast to the effects of cyclic AMP, raised levels of cyclic GMP can potentiate the antigen-induced release of histamine from human lung tissue. If, as is believed, the effect of increasing cyclic AMP levels is to inhibit the release of histamine and of raising cyclic GMP levels is to potentiate histamine release¹⁻³, the observed pharmacological effect of any agent inhibiting the hydrolysis of both nucleotides is likely to be related to the resultant change in the balance of the two nucleotide levels.

We have therefore investigated the ability of known and novel anti-allergic compounds to inhibit cyclic AMP and cyclic GMP hydrolysis and to inhibit anaphylaxis. Table 1 shows the pharmacological potency of twenty-five compounds in inhibiting anaphylaxis in passively sensitised human lung tissue and/or rat skin. Table 1 also shows the concentrations of the various compounds required to inhibit the hydrolysis of both cyclic nucleotides by 50% (I_{50}) using a microsomal preparation from human lung, and the inhibition constants (K_i) using a microsomal preparation from sheep lung. Also shown are the ratios of I_{50} (human lung) and K_i (sheep lung) for the hydrolysis of cyclic AMP to that of cyclic GMP. Table 2 shows the regression analyses of the relevant terms. A very significant correlation exists between the ability to inhibit anaphylactic reactions and the ratio of I_{50} cyclic AMP to I_{50} cyclic GMP or K_i cyclic AMP to K_i cyclic GMP. Figure 1 illustrates one of these relationships, that is that between potency of compounds in the human lung and the I_{50} ratio determined in human lung microsomes. This result indicates that com-

Table 1 Inhibition of anaphylaxis and phosphodiesterase activity

Inhibitor*	Pharmacological potency: relative to DSG = 1.00		Human lung			Sheep lung		
	Human lung	Rat PCA	I_{50} cyclic GMP (M)	I_{50} cyclic AMP (M)	I_{50} cyclic AMP I_{50} cyclic GMP	K_i cyclic AMP (M)	K_i cyclic GMP (M)	K_i cyclic AMP K_i cyclic GMP
Theophylline ¹⁵	0.04	0.01	5.60×10^{-4}	1.90×10^{-3}	0.29	5.70×10^{-4}	4.85×10^{-4}	1.18
Papaverine ¹⁶	0.12	0.07	4.50×10^{-5}	5.20×10^{-5}	0.87	2.33×10^{-5}	2.97×10^{-5}	0.78
M+B 21231(40) ^{18†}	0.16	0.47	5.70×10^{-4}	6.40×10^{-4}	0.89	2.91×10^{-3}	5.31×10^{-4}	5.48
M+B 21703(19) ^{18†}	0.22	6.23	4.01×10^{-5}	4.45×10^{-5}	0.90	1.17×10^{-4}	3.57×10^{-5}	3.28
Sc 2964 ¹⁸	0.27	0.04	2.62×10^{-5}	2.85×10^{-5}	0.92	1.11×10^{-4}	4.06×10^{-5}	2.73
M+B 23268(18) ^{17†}	0.32	0.29	3.10×10^{-4}	1.67×10^{-4}	1.86	3.70×10^{-4}	2.84×10^{-5}	13.03
Indomethacin ¹⁸	0.74	0.01	6.10×10^{-5}	4.00×10^{-5}	1.50	6.86×10^{-4}	1.67×10^{-4}	4.11
Ketoprofen ¹⁹	0.82	0.01	5.60×10^{-3}	2.15×10^{-3}	2.60	4.96×10^{-3}	5.57×10^{-3}	0.89
Disodium cromoglycate ¹⁹	1.00	1.00	4.80×10^{-3}	6.20×10^{-3}	0.77	4.12×10^{-3}	5.46×10^{-4}	7.55
M+B 21378(7) ^{18†}	1.30	4.75	1.00×10^{-3}	1.90×10^{-4}	5.26	3.01×10^{-3}	5.83×10^{-4}	5.16
Sch. 15280 ²⁰	1.55	—	3.85×10^{-4}	1.80×10^{-3}	0.21	—	—	—
RO 20-1724/1(16) ^{21†}	2.55	—	1.70×10^{-3}	1.68×10^{-3}	1.01	—	—	—
SQ20009 ²²	3.45	—	2.15×10^{-4}	3.55×10^{-5}	6.42	5.99×10^{-5}	1.20×10^{-5}	5.00
M+B 22106(22) ^{17†}	4.67	5.33	1.60×10^{-4}	1.10×10^{-4}	1.46	1.42×10^{-3}	2.62×10^{-4}	5.42
A+H 7079 ²⁴	8.45	5.74	1.60×10^{-5}	2.83×10^{-6}	5.65	3.44×10^{-3}	2.41×10^{-4}	14.27
ICI 58301 ²⁵	9.00	0.05	6.75×10^{-4}	2.55×10^{-4}	2.65	7.17×10^{-3}	2.17×10^{-3}	3.30
Doxantrazole ⁷	13.20	0.24	—	—	—	5.88×10^{-5}	2.94×10^{-5}	2.00
M+B 25565 ²⁶	14.30	792.97	2.95×10^{-4}	1.57×10^{-5}	18.80	4.27×10^{-4}	5.13×10^{-6}	83.24
ICI 74917 ⁶	15.30	69.53	—	—	—	4.13×10^{-4}	3.53×10^{-5}	11.70
M+B 23231(27) ^{17†}	16.50	5.02	1.25×10^{-4}	2.57×10^{-5}	4.86	1.61×10^{-3}	1.98×10^{-4}	8.13
M+B 22948 ²⁷	17.40	10.59	1.25×10^{-4}	7.95×10^{-6}	15.70	1.66×10^{-4}	2.01×10^{-5}	8.26
A+H 7725 ²⁴	24.00	6.29	2.30×10^{-3}	2.42×10^{-4}	9.50	5.06×10^{-3}	3.17×10^{-4}	16.00
BRL 10833(87) ^{23†}	42.00	9.70	8.50×10^{-3}	7.90×10^{-4}	10.80	2.46×10^{-3}	1.49×10^{-4}	16.50
ICI 63197 ²⁸	—	0.02	—	—	—	7.66×10^{-5}	1.07×10^{-3}	0.07

Inhibition of anaphylaxis in the human lung was measured using macroscopically normal tissue specimens obtained at lobectomy. Lung parenchyma was prepared and sensitised as described before⁹. The effects of drugs on the release of histamine were determined by an incubation method¹⁰, using an extract of *D. farinae* as antigen. The drugs were added immediately before challenge. Histamine was assayed fluorimetrically¹¹. Potency of drugs relative to disodium cromoglycate was calculated by comparing the molar concentrations required to produce equivalent inhibition of histamine release (30–40%). In the rat, passive sensitisation of skin areas was produced by intradermal injection of *Nippostrongylus brasiliensis* antiserum. After 48 h rats were challenged intravenously with *N. brasiliensis* extract. The compounds were administered intravenously immediately before challenge. The effective dose of each compound was measured as the minimal concentration to give 100% inhibition of the cutaneous reaction (ID_{100}) in two rats. Potency of drugs relative to disodium cromoglycate was calculated as the ratio of ID_{100} disodium cromoglycate (M) to ID_{100} test drug (M). In preparation for phosphodiesterase assay human lung was chopped with a McIlwain chopper and then ground with sand. The resulting homogenate was centrifuged to obtain the 100,000g pellet which was resuspended in 0.1 M Tris buffer, pH 7.4. The assay system (0.2 ml) contained 0.16 μ M ³H-cyclic nucleotide, 4.5 mM MgSO₄ and microsomes (0.5–1.0 μ g per ml protein) in Tris buffer 0.1 M, pH 7.4. Addition of 5'-nucleotidase was found to be unnecessary. Enzyme activity was measured after 20 min at 37 °C as the rate of formation of tritiated products¹². Each compound was tested twice using five concentrations in duplicate. The I_{50} s were obtained graphically and averaged. Sheep lung microsomal fraction was prepared as for human lung and the inhibition of phosphodiesterase activity was measured as above, but using the modification described by Boudreau and Drummond¹³. This allows for the quantitative recovery of adenosine, guanosine and their metabolites, which are produced when assaying crude enzyme preparations or tissue homogenates. After the discovery of a calcium-dependent protein activator for bovine brain phosphodiesterase¹⁴, 0.5 mM calcium chloride was added to the assay medium to maximise phosphodiesterase activation. Substrate concentration was varied from 3.33 to 20 μ M. Values of K_i were calculated by double reciprocal plot, using not less than four independent determinations.

*Suffixes refer to the publication in which the compounds are described.

†A compound with a number in parentheses is exemplified by that number in patent claim or paper.

Table 2 Multiple regression analysis

Variables		No. of observations	Slope	Intercept	Student's <i>t</i> test	Correlation coefficient <i>R</i>	<i>P</i>
1	2						
log. pharm. pot. (lung)	log. <i>I</i> ₅₀ cyclic GMP	21	-0.26	0.75	1.26	0.278	NS
log. pharm. pot. (rat)	log. <i>I</i> ₅₀ cyclic GMP	18	-0.73	-2.98	1.94	0.436	< 0.1
log. pharm. pot. (lung)	log. <i>I</i> ₅₀ cyclic AMP	21	0.24	1.09	0.97	0.217	NS
log. pharm. pot. (rat)	log. <i>I</i> ₅₀ cyclic AMP	18	-0.14	0.64	0.31	0.078	NS
log. pharm. pot. (lung)	log. <i>K</i> ₁ cyclic GMP	21	-0.16	-0.27	0.59	0.134	NS
log. pharm. pot. (rat)	log. <i>K</i> ₁ cyclic GMP	21	-1.11	-4.39	2.99	0.567	< 0.01
log. pharm. pot. (lung)	log. <i>K</i> ₁ cyclic AMP	21	0.30	1.28	1.11	0.247	NS
log. pharm. pot. (rat)	log. <i>K</i> ₁ cyclic AMP	21	0.16	0.34	0.34	0.078	NS
log. pharm. pot. (lung)	log. <i>R</i> (<i>K</i> ₁)*	21	1.23	0.53	3.86	0.663	< 0.001
log. pharm. pot. (rat)	log. <i>R</i> (<i>K</i> ₁)*	21	1.82	-1.34	5.24	0.769	< 0.001
log. pharm. pot. (lung)	log. <i>R</i> (<i>I</i> ₅₀)*	21	1.25	0.19	5.68	0.794	< 0.001
log. pharm. pot. (rat)	log. <i>R</i> (<i>I</i> ₅₀)*	18	1.84	-0.89	3.30	0.637	< 0.01

The data in Table 1 were submitted to multiple regression analysis using a DEC System PDP-10 computer. NS, not significant at the 0.1 level.

* $R(I_{50}) = I_{50}$ cyclic AMP/ I_{50} cyclic GMP human lung; $R(K_1) = K_1$ cyclic AMP/ K_1 cyclic GMP sheep lung.

pounds which are more potent inhibitors of the anaphylactic reaction inhibit the hydrolysis of cyclic GMP more effectively than that of cyclic AMP. We find that the correlation does not hold for the supernatant enzyme activity from human lung (unpublished work of C.J.C.). This suggests that the effect of the cyclic nucleotides takes place in a membrane environment.

Relationships of lesser significance are obtained between inhibition of cyclic GMP hydrolysis and pharmacological potency in the rat. These results indicate that although the balance between the two cyclic nucleotides seems to be critical in determining the anti-allergic activity of a compound, cyclic GMP seems to be the more important factor in the rat PCA test.

In some cases it seems to be difficult to reconcile the drug concentrations effective in inhibiting histamine release from human lung fragments with those which inhibit human lung microsomal phosphodiesterase. The apparent discrepancy could possibly be related to the lipophilic properties of the drugs concerned and the sites of the enzymes involved. Direct measurement of drug-induced changes in cyclic nucleotide levels within the membranes of target cells would provide a better understanding of their interrelationship. Further work is planned in an attempt to elucidate these points.

Cyclic AMP and cyclic GMP have been suggested to

have antagonistic actions as intracellular messengers and regulators in certain biological systems³⁰. There have been several reports of control systems of this type³¹⁻³³. It seems likely that such a mechanism is operating here and that the selectivity of the inhibitor between the two cyclic nucleotide phosphodiesterases governs the biological activity. The implication of our results is that histamine release is reduced more effectively when cyclic GMP levels are increased with respect to cyclic AMP levels. This result is in opposition to the concept discussed by Austen *et al.*¹⁻³, but they present only circumstantial evidence, linking increased cyclic GMP levels with increased histamine release. No workers, as far as we know, have measured the levels of both nucleotides in human lung simultaneously. It is interesting that McElroy and Philip³⁴ also find that the ratio of K_1 s but not the individual K_1 for each cyclic nucleotide correlates with inhibition of ADP-induced aggregation in platelets.

The fact that highly significant correlations were obtained between selective inhibition of phosphodiesterase activity and pharmacological potency *in vitro* and *in vivo* tests, involving different species and different tissues, may reflect a basic common mechanism of fundamental importance in allergic reactions.

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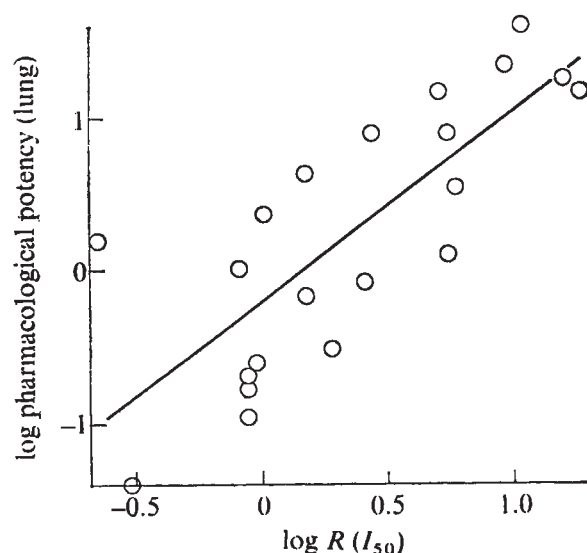
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Fig. 1 The logarithm of the pharmacological potency plotted against the logarithm of the I_{50} ratio. The straight line was obtained by regression analysis. Data are from human lung.



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Sperm-associated sialyltransferase activity

FERTILISATION begins with a specific adhesion between sperm and egg and culminates with the fusion of the male and female pronuclei. In mammals, sperm seem to recognise the zona pellucida surrounding the egg¹. This adhesive interaction is the first of many that will take place during subsequent embryogenesis. In molecular terms, these later cell-cell interactions are not generally understood, and the mechanism of sperm-egg recognition is unknown. Conceptually, advances in this area have been rare since Lillie presented the fertilisin hypothesis, and even this has been questioned². There is evidence to support a model for intercellular recognition that utilises the molecular complementarity inherent in cell surface glycosyltransferases and their respective acceptor molecules^{3,4}. The transferases catalyse the addition of sugars, from sugar nucleotides, to the non-reducing termini of saccharide acceptors. The enzymes are highly specific for both the donor sugar nucleotide and the acceptor, usually an oligosaccharide. For example, sialyltransferases are the class of glycosyltransferases that transfer sialic acid (*N*-acetylneuraminic acid) from cytidine monophosphate-*N*-acetylneuraminic acid (CMPNAN) to either glycoprotein, glycolipid, or free oligosaccharide acceptors⁴. Glycosyltransferases have been reported on the external surfaces of many different cell types and, in some cases, enzyme-substrate complexes between the transferases and their acceptors may be instrumental in the cellular recognition that occurs (reviewed in ref. 5). We report here a study of mouse sperm-egg interactions in terms of the transferase-acceptor hypothesis.

CMPNAN:asialofetuin sialyltransferase activity was associated with intact sperm, although no endogenous acceptors for the enzyme were detected. Autoradiographic assays supported the absence of endogenous surface sialyltransferase activity on intact sperm, but showed moderate activity on mouse ova from C57Bl females superovulated by injection of 10 U of gonadotropin from pregnant mare serum (Sigma) and, after 48 h, by injection of 10 U of human chorionic gonadotropin (Sigma). Ova were collected from the dissected uterine ampullae 16 h after injection of the human chorionic gonadotropin. When sperm and ova were incubated together with the sialic acid donor, additional dense grain patches were found on the ova surfaces. These accumulations were often associated with penetrated sperm (Fig. 3). Ova incubated identically, but without sperm, did not show these patches (Fig. 2).

Sperm were obtained from the cauda epididymis and vas deferens of mature male C57Bl mice by dissection and collection in a saline solution, pH 7.3, containing (in g l⁻¹): NaCl, 7.13; glucose, 1.0; KCl, 0.4; phenol red, 0.004, and HEPES, 2.35. This sperm suspension was centrifuged once at 10g for 4 min to sediment tissue debris. The supernatant sperm suspension was then washed three times by centrifugation (500g, 6 min) and resuspended in the saline solution described above.

The first, 500g supernatant from the sperm preparation and the corresponding sperm pellet contained approximately equivalent levels of sialyltransferase activity toward the exogenous acceptor asialofetuin. This supernatant enzyme was probably derived from seminal fluid, because levels of activity decreased in the next two supernatants while the activity of the sperm pellet remained constant. The activity observed with intact sperm may result from a sialyltransferase that is initially in seminal fluid and, secondarily, adsorbed on to sperm rather than being an intrinsic part of the sperm plasma or acrosomal membrane. In the conditions described in the legend to Fig. 1, and with 10⁶ sperm as the

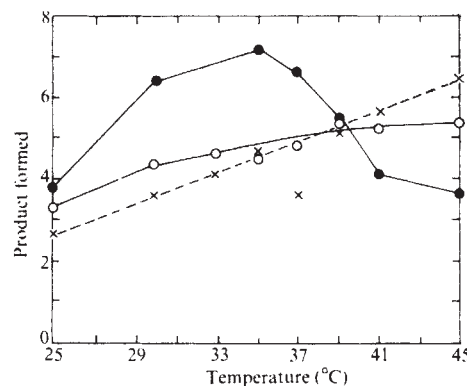


Fig. 1 Reaction rates of murine sperm enzymes as a function of temperature. Sialyltransferase activity toward asialofetuin, in pmol ³H-sialic acid transferred per h per 10⁶ sperm (●), acid phosphatase activity, in μmol *p*-nitrophenol formed per h per 10⁶ sperm (○) and β-galactosidase activity, in μmol *p*-nitrophenol formed per h per 10⁶ sperm (×). Asialofetuin was prepared from fetuin (Sigma) by mild acid hydrolysis in 0.1 N H₂SO₄ for 75 min at 80 °C. The hydrolysate was adjusted to pH 7.3 with 0.25 N NaOH and dialysed exhaustively in 0.01 M HEPES, pH 7.3, at 20 °C (5 ml hydrolysate against 3 × 700 ml dialysate over at least 24 h). Similarly, ³H-NAN was obtained from CMP-³H-NAN (New England Nuclear) by mild acid hydrolysis in 0.3 N HCl for 15 min at room temperature. The hydrolysate was neutralised with 0.3 N NaOH and assayed by descending paper chromatography on Whatman No. 1 paper with 95% ethanol and 1 M ammonium acetate (5:2) as a solvent. Quantitative cleavage of the CMP-³H-NAN occurred in a concentrated solution (55 μl CMP-³H-NAN at 2.33 Ci mmol⁻¹ was desiccated and hydrolysed in 25 μl of HCl). For sialyltransferase assays 0.125 nmol of CMP-³H-NAN, 1.25 mg asialofetuin and 25 μl of 10 mM MgCl₂ were desiccated in a small test tube (neither MgCl₂ nor MnCl₂ had any significant inhibitory or stimulatory effects). Reactions were initiated by adding 25 μl of the washed, intact sperm suspension to each tube before incubation at 35 °C. The reactions were stopped by the addition of 10 μl of ice-cold 0.25 M EDTA and by immediate chilling to 0 °C. Although cations were not required for the sialyltransferase reaction, the addition of EDTA consistently lowered electrophoretic background activities. The amount of ³H-sialic acid transfer was determined by high voltage electrophoresis of 20 μl of the reaction mixture using methods described before⁷. Transfer to endogenous acceptors was determined by omitting asialofetuin from the reaction tubes. For acid phosphatase and β-galactosidase assays, 25 μl of the washed sperm suspension was added to 200 μl of a 50 mM citrate buffer, pH 5.1, containing either *p*-nitrophenyl phosphate or *p*-nitrophenyl β-D-galactopyranoside (both at 1 mg ml⁻¹, Sigma). After 1 h, the reactions were terminated by the addition of 1.5 ml ice-cold 0.02 M NaOH and the values of A₄₁₀ were recorded and converted to μmol of product formed using a conversion factor of 0.245 Δ units per μmol free *p*-nitrophenol.

enzyme source, the rate of transfer was 4.5 pmol of sialic acid transferred per h at 35 °C. This rate was linear with time for at least 150 min. If asialofetuin was omitted from the reaction mixtures there was no detectable endogenous activity with washed sperm.

Figure 1 shows that the sialyltransferase activity on intact sperm was highest at 35 °C, the approximate temperature of the mouse testis⁶, and that it decreased at higher or lower temperatures. This contrasts with sperm acid phosphatase and β -galactosidase, for which activity increases with temperature up to at least 45 °C.

When ova alone were incubated with CMP-³H-NAN for 1 h and examined autoradiographically, they showed a definite endogenous incorporation that was significantly above background but diffusely distributed on the ova and surrounding cumulus cells (Fig. 2). Ten ova were assayed in this fashion in three separate experiments, and all showed the same pattern. By contrast, when 12 ova, in three experiments, were incubated with sperm for 1 h, all the eggs contained at least one very dense patch of silver grains in addition to the diffuse pattern seen on all eggs. Figure 3a shows a brightfield micrograph of an egg incubated with sperm and processed autoradiographically. This egg has three, clear deposits of silver grains in which the density of the grains is much greater than that of the surrounding area. The same egg is shown under phase contrast in Fig. 3b. Two of the dense grain deposits (arrows) in Fig. 3a are clearly associated with sperm that have penetrated the egg and whose tails are visible in phase contrast in Fig. 3b (arrows). The third patch of silver grains on the egg (left-most edge of the egg in Fig. 3a) cannot be linked unambiguously to a single sperm although there are several possible candidates visible when the actual specimen is viewed at a number of focal planes in phase contrast. Grain accumulations are also seen on cumulus cells of eggs incubated with sperm (Fig. 3a) but not on cumulus cells of eggs incubated without sperm (Fig. 2). The localised, spatial correlation between grain patches and sperm indicate that the transfer is mediated by sperm-associated and not soluble enzyme activities.

In these incubations, hydrolysis of CMP-³H-NAN did occur but the extent of hydrolysis did not exceed 17% of the input sugar nucleotide. Further, this level of free ³H-NAN cannot account for any of the activities reported here because, when ³H-NAN was substituted for CMP-³H-NAN at identical concentrations (0.125 nmol per 25 μ l) and

Fig. 2 Brightfield micrograph of a mouse egg incubated in CMP-³H-NAN and developed autoradiographically. There is a definite incorporation of radioactivity above background levels on the egg as well as on cumulus cells partially surrounding the egg, and this incorporation is distributed randomly. The bar represents 25 μ m.

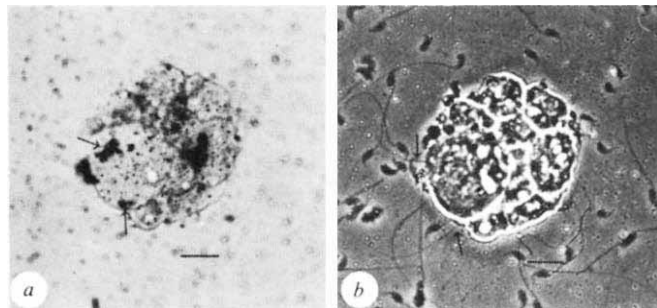


Fig. 3 Mouse egg incubated as in Fig. 2 but in the presence of washed sperm. *a*, Brightfield micrograph showing three dense accumulations of grains over the egg and several accumulations over the cumulus cells. These patches of grains are in addition to the general, evenly distributed incorporation seen in eggs incubated without sperm. Arrows indicate two grain patches on the egg. *b*, Phase contrast micrograph of the identical field shown in *a*. Here the sperm are easily seen. Arrows show the tails of sperm that have penetrated the egg in precisely those areas where two of the grain accumulations can be seen (arrows) in *a*. The bar represents 35 μ m. Autoradiographic assays were performed using concentrations of CMP-³H-NAN identical to those described in the legend to Fig. 1, except that the contents of the incubation tubes containing either ova alone or ova with washed sperm were first placed on a slide kept on a humidified chamber for the duration to the incubation. The reactions were stopped by gentle rinsing of the ova, or ova with sperm, with saline followed by fixation in 10% formalin. The slides were then stained lightly with haematoxylin and processed by standard autoradiographic techniques.

specific activities (prepared as described in the legend to Fig. 1), there was no sialic acid transfer to asialofetuin by sperm.

These data show that a CMPNAN:asialofetuin sialyltransferase is associated with mouse sperm and suggest that intact sperm can transfer sialic acid to acceptors on egg membranes. In the absence of added CMPNAN, it is possible that an enzyme-substrate complex would be more stable and could be one of the mechanisms whereby a sperm recognises and adheres to an egg. Although we have no data to suggest that the enzyme activity expressed by intact sperm results from intrinsic, as opposed to adsorbed, transferases, even an adsorbed enzyme could function in the suggested manner.

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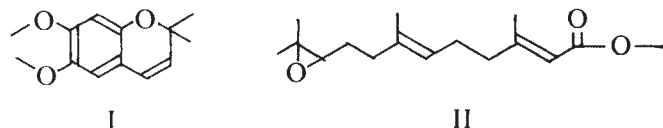
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Precocene II inhibits juvenile hormone biosynthesis by cockroach corpora allata *in vitro*

BOWERS *et al.*¹ reported that a chromene derivative isolated from plants of the genus *Ageratum* interferes with the development of insects from several orders. They named the most active component of the plant extract precocene II (I), because exposure of young larval stages of test insects to the compound leads to precocious metamorphosis into unviable or moribund miniature adults. Also, exposure of

adult females of several insect species causes sterility by preventing normal vitellogenic development of the oocytes, and in at least one case¹ this effect can be overcome by hormone replacement therapy with C_{16} juvenile hormone ($C_{16}JH$: (II)). These effects suggest that precocene II acts



antagonistically to the natural JHs which are responsible for the proper suppression of metamorphosis in larval insects², and for the activation of ovarian growth and other reproductive phenomena in adult females of most insects³. Precocenes constitute a new type of insect growth regulator, and have been referred to as 'fourth generation insecticides'. We envisaged two ways *a priori*, in which precocene might act in the whole animal: either by blocking the biosynthesis of JHs, or by blocking the action of natural JHs, that is, their safe transport through the haemolymph followed by effective action at the various target sites. We have concentrated on one aspect of the former possibility, and have investigated whether or not active corpora allata from adult females of the cockroach *Periplaneta americana* are directly inhibited by exposure to precocene II *in vitro*.

We used minor modifications of the quantitative assay for inhibitors of JH biosynthesis developed by Pratt and Finney⁴, which is based on (1) the predictability of synthetic activity in corpora allata taken from adult mated females at specified times during the gonotrophic cycle, (2) the incorporation of label from methyl- ^{14}C -methionine into $C_{16}JH$, and (3) the linearity of this incorporation for at least 5 h *in vitro*^{6,7}. Because of natural increases in the reproduction *in vitro* to concentrations of precocene II of 10^{-4} M and corpora allata are now maximally active 0.75 d after the mid-brown stage of ootheca formation⁸, affording rates of $C_{16}JH$ biosynthesis which range from 28 to 45 pmol per pair of glands per h. Figure 1 shows that spontaneously active corpora allata can be inhibited rapidly by exposure *in vitro* to concentrations of precocene II of 10^{-4} M and higher; moreover, the inhibition of $C_{16}JH$ biosynthesis measured in these standard conditions is essentially complete at concentrations in excess of 10^{-3} M. The influence of precocene II on $C_{16}JH$ biosynthesis was quantitatively indistinguishable from its effect on total ester production (methyl farnesoate + $C_{16}JH$). This level of inhibitory activity is approximately commensurate with that of some propynyl and methylenedioxyphenyl ethers previously examined but much lower than that of those methylenedioxyphenyl ethers which bear a strong structural resemblance to natural JHs and have intrinsic JH-mimicking activity⁴. There is no doubt that precocene II can act as a powerful direct inhibitor of the corpus allatum which suggests that analogous structures may have this activity at much lower concentrations, as is the case with methylenedioxyphenyl series.

That the observed level of methyl farnesoate in the glands treated with precocene II was reduced in proportion to the reduction in the rate of $C_{16}JH$ biosynthesis might suggest that the site of action is earlier than the final epoxidative step in the biosynthetic pathway. Failure to accumulate methyl farnesoate in the presence of monooxygenase inhibitor, however, might also occur if internal feedback mechanisms prevented the overproduction of farnesenic acid and hence methyl farnesoate⁴.

To investigate this possibility, we have made use of the fact that addition to the culture medium of the presumed precursor farnesenic acid results in a significant stimulation of the rate of $C_{16}JH$ biosynthesis, regardless of the spontaneous level of activity of the glands. The exogenous

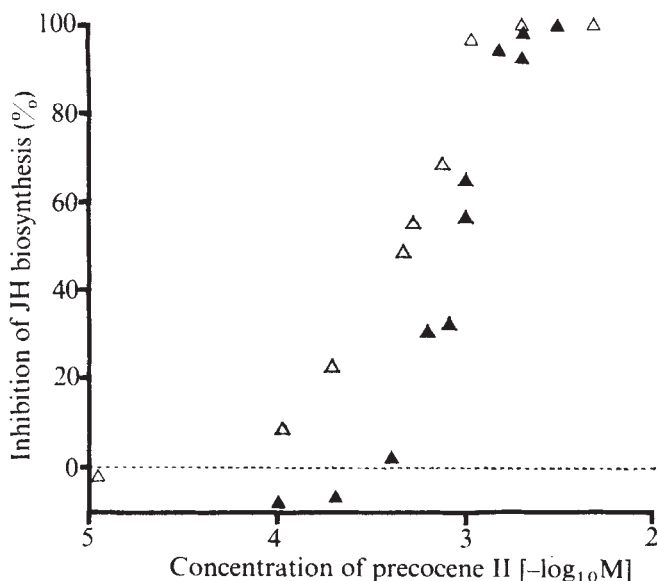


Fig. 1 Effect of precocene II concentration on its ability to inhibit biosynthesis of $C_{16}JH$ *in vitro* during a 3-h incubation with label after a 30-min preincubation. Δ , Response of corpora allata synthesising spontaneously at their maximum physiological rate; $\blacktriangle$, response of glands taken at a time of minimum spontaneous activity but stimulated with optimum concentration of exogenous farnesenic acid. Each point represents the performance of four, six or eight pairs of glands. The two glands from each member of the group were bilaterally randomised between control and experimental tubes, and preincubated in 0.1 ml of sterile augmented medium TC-199 for 30 min with or without precocene II, at 30 °C; the medium was removed and replaced with the same medium fortified with methyl- ^{14}C -methionine to final specific radioactivities of 21–23 mCi mmol⁻¹ in different experiments. After 3 h incubation with labelled methionine the entire contents of the tubes were brought to pH 4.5 with an equal volume of 1% disodium ethylene-diamine-tetracetate, and analysed for the methyl farnesoate and $C_{16}JH$ that had been biosynthesised^{6,9}. Appropriate sections of the chromatograms were counted in 11 ml of 7.3% naphthalene, 0.7% butyl-PBD, 0.04% glacial acetic acid, 11% water, in 1:4 dioxan, after vigorous shaking for 1 h and being left overnight in the dark in a Wallac 8100 LSC set for double-label 3H , ^{14}C counting.

3H -farnesenic acid is incorporated efficiently into the newly synthesised $C_{16}JH$ (refs 8 and 9). Moreover, large quantities of methyl farnesoate accumulate within the glands in these conditions because of a limited epoxidative capacity. Apparently there is no rapid end-product inhibition of the *O*-methyl transferase. Thus by carrying out trials with precocene II in the presence and absence of exogenous farnesenic acid we were able to determine whether the inhibitor has an effect on JH biosynthesis subsequent to the point of entry of farnesenic acid into the biosynthetic pathway. The optimum concentration of farnesenic acid for stimulation of $C_{16}JH$ biosynthesis by corpora allata from the locust *Schistocerca gregaria* is known to be $2\text{--}2.5 \times 10^3$ M (ref. 10). Glands from *Periplaneta americana* respond optimally to slightly higher concentrations (unpublished results of G.E.P.) and so a concentration of $4\text{--}4.5 \times 10^{-3}$ M was used. By killing animals between ovulation and ootheca formation (2.75 d after the mid-brown stage of the previous ootheca) we obtained corpora allata exhibiting low spontaneous rates of $C_{16}JH$ biosynthesis (2–5 pmol per pair of glands per h⁵); in the presence of optimal concentrations of farnesenic acid, these glands afforded rates of $C_{16}JH$ biosynthesis of 40–55 pmol per pair of glands per h, and total esterification rates of 60–95 pmol per pair of glands per h.

Analysis of the isotope incorporation ratio in the $C_{16}JH$ indicated that in these 'stimulated' conditions, 91–98% of

the hormone is made from exogenous acid, so that the assay approximates closely a test for the ability of precocene II to inhibit esterification and epoxidation reactions at the end of the biosynthetic pathway. Figure 1 shows that we obtained a very similar result for the inhibition of both spontaneous and farnesenic-acid-dependent synthesis; moreover, the rate of esterification of the ^3H -farnesenic acid (that is the rate of formation of both methyl farnesoate and C_{16}JH) showed the same sensitivity to precocene II inhibition as did the terminal epoxidation to the hormone. One interpretation of these results is that the effect of precocene II is manifested most importantly at the penultimate esterification stage in the biosynthetic pathway. The results shown in Fig. 1 also imply that the glands are slightly less sensitive to precocene II when assayed in the presence of optimum farnesenic acid concentrations, than when assayed at the lower rates of flux through the last two stages which obtain in conditions of maximum spontaneous activity in the absence of added precursor acid. A better understanding of this apparent protecting effect of exogenous farnesenic acid might afford a clue to the molecular mode of action of precocene II.

To see whether precocene II has a progressive effect on JH biosynthesis, we examined the effect of varying the duration of the non-radioactive preincubation on the ability of critical concentrations of precocene II to inhibit hormone radiobiosynthesis. We maintained sterile procedures throughout the experiment, and reduced the duration of the labelled incubation to 2 h. Such a reduction in labelling time results in an underestimation of the actual rates of biosynthesis, because of the time-lag in the attainment of full isotopic equilibrium in the newly synthesised hormone from methyl- ^{14}C -methionine⁶. The results presented in Table 1 suggest that there is an approximate doubling in the effectiveness of precocene II over the 6 h of our study. This simple interpretation however, assumes that precocene II has no effect on the radiochemical lag period. If precocene progressively inhibits either the uptake or metabolism of methionine so as to increase the time taken for the methyl-donor substrate pool serving the *O*-methyl transferase to reach isotopic equilibrium, this would manifest itself as an apparent enhancement of the inhibition of C_{16}JH biosynthesis. Direct measurements have not been reported of intracellular pools of methionine or *S*-adenosyl methionine¹¹ in the corpora allata of any species. On the other hand, our results make it unlikely that the glands rapidly inactivate or adapt to the inhibitor.

Although precocene II inhibits C_{16}JH biosynthesis in the corpora allata of adult female *P. americana* only at relatively high concentrations compared with some other

compounds tested⁴, its action on the corpus allatum is interesting for two reasons. First, it is a natural product, and second it has dramatic 'anti-juvenile hormone' effects in several insects, notably hemipterans¹. Unfortunately, the chemical identity of the natural juvenile hormone of sensitive hemipterans has not yet been established. Precocene II has only weak activity in adult female *P. americana* as an 'anti-juvenile hormone', which is in accord with the high ID_{50} *in vitro* reported here, and could also be a result of high rates of metabolism in this species. Although we have found that precocene II is an effective inhibitor of C_{16}JH biosynthesis in isolated corpora allata, this does not preclude the possibility that it also interferes with other parts of the juvenile hormone control pathway, such as haemolymph transport and target site recognition, which have yet to be directly investigated. It also remains to be discovered whether precocenes are specific inhibitors of the penultimate step in JH biosynthesis, or alternatively inhibit biosynthesis through nonspecific or indirect actions on cellular metabolism. We do not exclude the possibility that precocenes are general metabolic depressants, or inhibit the synthesis of enzymes in the JH biosynthetic pathway which have a rapid turnover.

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Table 1 Influence of preincubation time on percentage inhibition of JH biosynthesis by precocene II *in vitro*

Preincubation time (h)	Precocene II 0.25 mM (%)	concentration 0.5 mM (%)
0	20*	20
0.5	20	52
2	20	60
4	71	91
6	64	98

Groups of spontaneously active corpora allata, bilaterally randomised into control and experimental sets, were preincubated in standard culture medium with or without precocene II for up to 6 h. Incubation medium was aspirated off and replaced with the same medium plus labelled methionine. After 2 h further incubation, the rates of production of C_{16}JH were determined by addition of nonradioactive carrier, extraction, thin-layer chromatography and liquid scintillation counting. The percentage inhibitions shown reflect the performance of not less than six pairs of corpora allata in each case.

*Observed inhibitions of less than 20% are not significantly different from zero.

Facultative blood-brain barrier and neuronal adaptation to osmotic stress in a marine osmoconformer

IN most vertebrate and higher invertebrate animals cellular functions are critically dependent on the relative stability of the chemical composition of the body fluids, a requirement epitomised by Claude Bernard's dictum: "la fixité du milieu intérieur c'est la condition de la vie libre"¹. In higher vertebrates^{2,3}, and in insects⁴, but not apparently other invertebrates^{5,6}, the ionic homeostasis of the neuronal environment is augmented by extremely effective blood-brain barrier systems. The nerve cells of euryhaline osmoconformers, however, are exposed to considerable fluctuation in the osmotic and ionic concentrations of the body fluids⁷. As an example we studied the serpulid worm, *Mercierella enigmatica* (Fauvel), which occurs in waters in which the salinities vary from 1 to 55‰ (refs 8-10) and which can withstand relatively rapid variations in the osmotic concentration of the blood of between 43 and 1,620 mOsm (ref. 11). We report here that in spite of such extreme variations

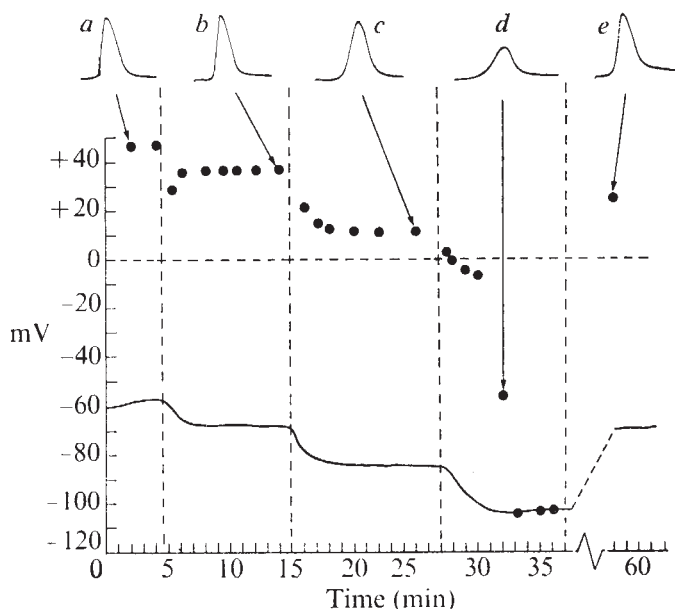


Fig. 1 Effects of isosmotic dilution on action and resting potentials. Isosmoticity (1,024 mOsm) was maintained by addition of sucrose to the physiological saline. The intracellular recordings were made with conventional glass microelectrodes (10–20 M Ω tip resistance) from the paired giant axons (30 μ m in diameter) in pinned, dissected worms. The axons were stimulated with insulated silver wire electrodes. The standard saline used was based on the ionic and osmotic concentration of the blood of seawater-adapted animals¹⁴ and had the following composition, in mM: Na⁺, 482.3; K⁺, 30; Mg²⁺, 77; Ca²⁺, 31; SO₄²⁻, 26; Cl⁻, 663.8; OH⁻, 12.5 and PIPES, 7.5 (pH 6.9). *a*, Std saline only; *b*, 50% saline in sucrose; *c*, 25% saline in sucrose; *d*, 10% saline in sucrose; *e*, Std saline.

in osmotic and ionic concentration, the axons of *Mercierella* function using ionic mechanisms which seem essentially similar to those of other marine invertebrates, such as the giant axons of the squid¹² or the polychaete, *Myxicola infundibulum*¹³. The resting potential approximates to an ideal potassium electrode (with 58.8 mV alteration for decade change in [K⁺]_o), the action potentials are sodium-mediated (exhibiting a 55.8 mV alteration for decade change in [Na⁺]_o) and are blocked by externally applied tetrodotoxin at about 10⁻⁷ M.

The axonal responses to saline diluted with isosmotic sucrose differed markedly from those observed during exposure to hyposmotic (distilled water-diluted) saline. Isosmotic dilution caused a significant hyperpolarisation, rapid decline in the overshoot of the action potential and eventual conduction block (Fig. 1). The hyperpolarisation probably results from dilution of the external potassium which, at 30 mM, is in a sensitive part of the Nernst slope. The decline in overshoot results from the reduced concentration of sodium ions at the axon surfaces. These responses differ from those observed during hyposmotic dilutions, when axonal function persisted in preparations exposed to dilutions down to 1.0% (Fig. 2) or, in other experiments, to 0.5%. Action potentials also continued in diluted, sodium-free saline (Fig. 2). In contrast, exposure to diluted sodium-free saline caused a rapid decline in overshoot in isosmotic conditions.

Potassium depolarisation of the axon membrane was critically dependent on the osmotic concentration of the bathing medium. Rapid axonal depolarisation occurred in isosmotic saline (Fig. 3). The kinetics of this potassium depolarisation indicate that the inward and outward movements of this cation, between the axon surface and the bathing medium, occurred with half times of 31.0 $\pm$ 3.1 and 32.2 $\pm$ 3.1 s ($n=6$), respectively. In hyposmotic saline, however, no appreciable

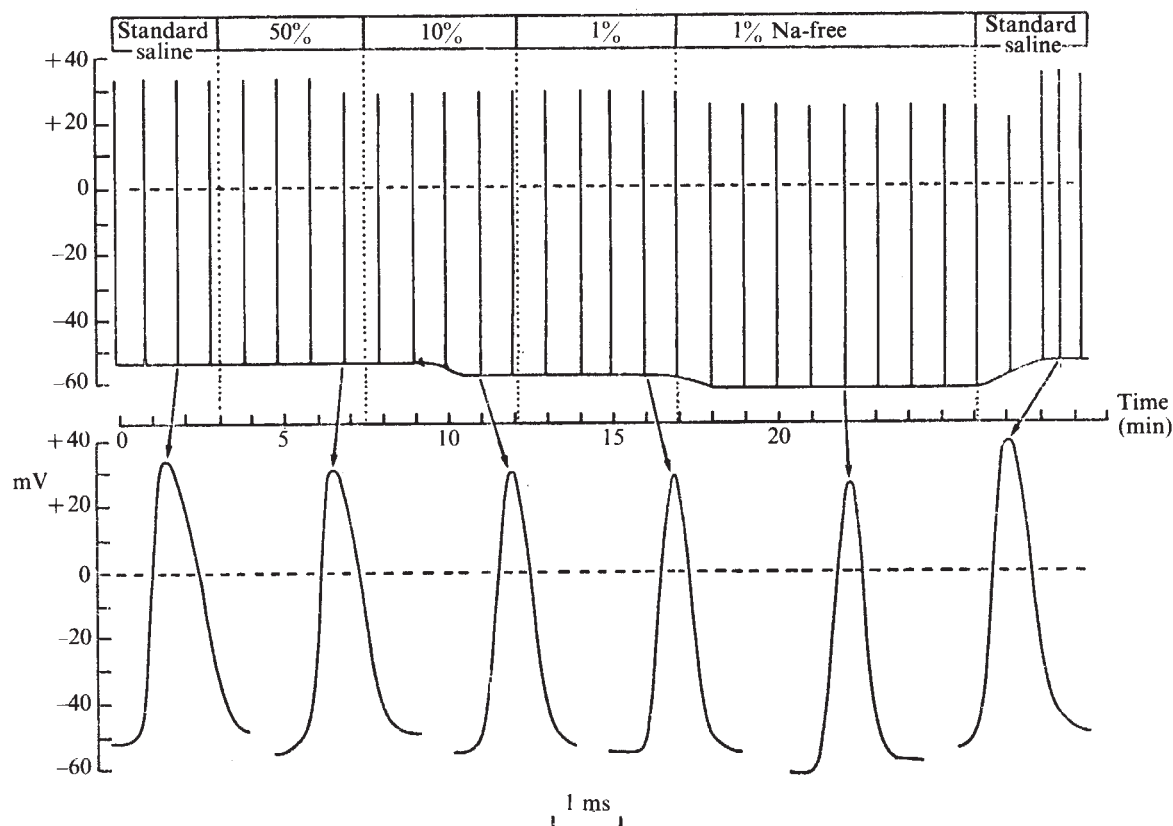


Fig. 2 Effects of hyposmotic dilution on axonal action and resting potentials. Normal physiological saline (1,024 mOsm) was diluted with distilled water down to 1% strength. Sodium-free saline was prepared by substituting Tris ions for Na⁺. The potentials were recorded by a transient signal processor and displayed on a chart recorder.

axonal depolarisation was seen after an increase in the external potassium concentration (Fig. 3).

The absence of potassium depolarisation and the lack of effect of sodium-free saline in hyposmotic conditions suggest that a drastic change in accessibility of the axon surfaces occurs during dilution of the bathing medium. This isolation of the immediate fluid environment of the axons was readily reversible, and was abolished when sucrose was added to the bathing medium to restore isosmotic conditions.

Mercierella can survive in distilled water. Axons from individuals adapted to such conditions were able to function in standard saline diluted to 10% strength (Fig. 4a). Exposure to sodium-free 10% strength saline caused a rapid loss of axonal function, which indicates that the axon surfaces are accessible in hyposmotically adapted animals. In these conditions further dilution of the bathing medium did not reduce access to the axon surfaces, as exposure to 1% standard strength saline resulted in a rapid decline in the overshoot and irreversible conduction block.

The structural basis of the osmotically-induced diffusion barrier is uncertain. Electron micrographs show that the giant axon is wrapped by narrow glial processes, which are associated with one another by occasional gap junctions, and that this glial-axonal complex is surrounded by extracellular material containing collagen-like fibres. The glial coverage of the axon seems to be incomplete, even in hyposmotic media, so that in these glial-depleted areas the axon abuts directly on to the extracellular material. This organisation is difficult to reconcile with a glial diffusion barrier. Furthermore, the absence of appreciable potassium depolarisation in hyposmotic conditions (Fig. 3) might argue against a cellular barrier, as restricted intercellular access through a peripheral glial layer would be expected to produce relatively large extraneuronal potential changes resulting from depolarisation of the outwardly-directed cell membranes as is the case in insect¹⁵ and crustacean¹⁶ perineuria. Alternatively the osmotically-induced restriction to the access of water-soluble cations might result

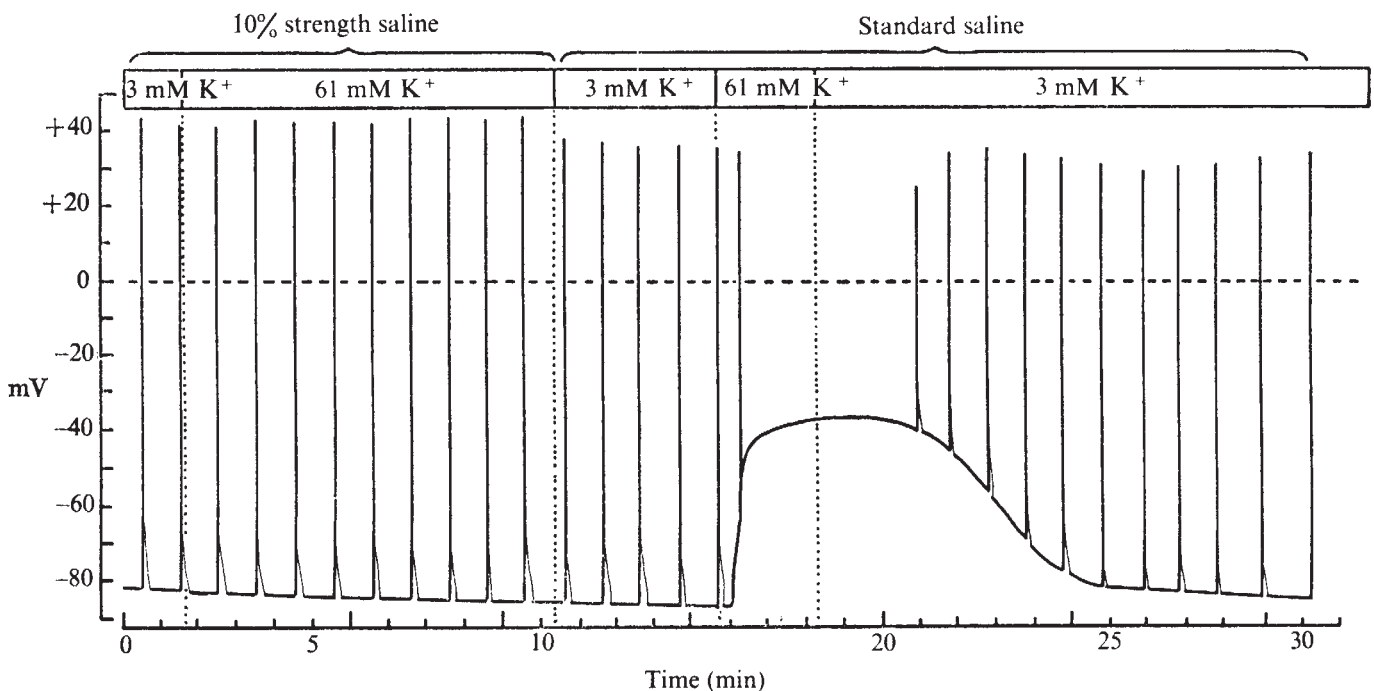
from changes in the permeability properties of the extracellular matrix which surrounds the axon surfaces.

The accessibility and ability of the axons of hyposmotically-adapted individuals to function at reduced $[Na^+]_o$ (48.2 mM) contrasts with the situation in the axons of seawater-adapted animals. In the latter the axons are protected during hyposmotic stress, and exposure to 10% standard strength sodium (48.2 mM) in isosmotic conditions causes conduction block (Fig. 4b). This indicates that the axons can adapt to conduct action potentials at drastically reduced external sodium concentrations.

Adapted axons bathed with 10% strength saline showed smaller overshoots (20.8 ± 1.8 mV, $n = 5$, compared with 38.1 ± 1.1 mV, $n = 20$) and larger resting potentials (69.8 ± 1.5 mV, $n = 5$, compared with 53.6 ± 1.4 mV, $n = 20$) than unadapted axons in normal saline. The total amplitude of the action potential was thus virtually unchanged (Fig. 4b). The resting potentials of adapted axons in 10% saline (3.0 mM K^+) were significantly smaller than those of unadapted axons in normal saline containing 3.0 mM K^+ (96.8 ± 2.2 mV, $n = 5$). This suggests that hyposmotic adaptation involves a reduction in $[K^+]_i$ and/or a decrease in potassium permeability of the axon membrane. Similarly, the ability of adapted axons to produce overshooting action potentials at a tenth of the standard sodium concentration (48.2 mM) implies that adaptation involves an appreciable reduction in $[Na^+]_i$. For example, if the sodium selectivity of the active membrane was similar to that of unadapted axons (55.8 mV for decade change in $[Na^+]_o$), then with $[Na^+]_o = 48.2$ mM and an overshoot of 20.8 mV it can be calculated that $[Na^+]_i$ would be approximately 21 mM, compared with 90 mM in unadapted axons bathed in normal saline.

Our observations indicate that two physiological mechanisms are involved in hyposmotic adaptation of this axon. Immediate protection from hyposmotic stress is achieved by a facultative blood-brain barrier which effectively isolates the axon from the diluted body fluids. This isolation enables the axons to adjust more slowly and, eventually, to function when bathed directly

Fig. 3 Effects of increased potassium (61 mM) in hyposmotic (10%) and normal saline. The potassium concentration of normal saline (30mM) was reduced to 3 mM to maintain similar concentrations to that in 10% standard strength saline.



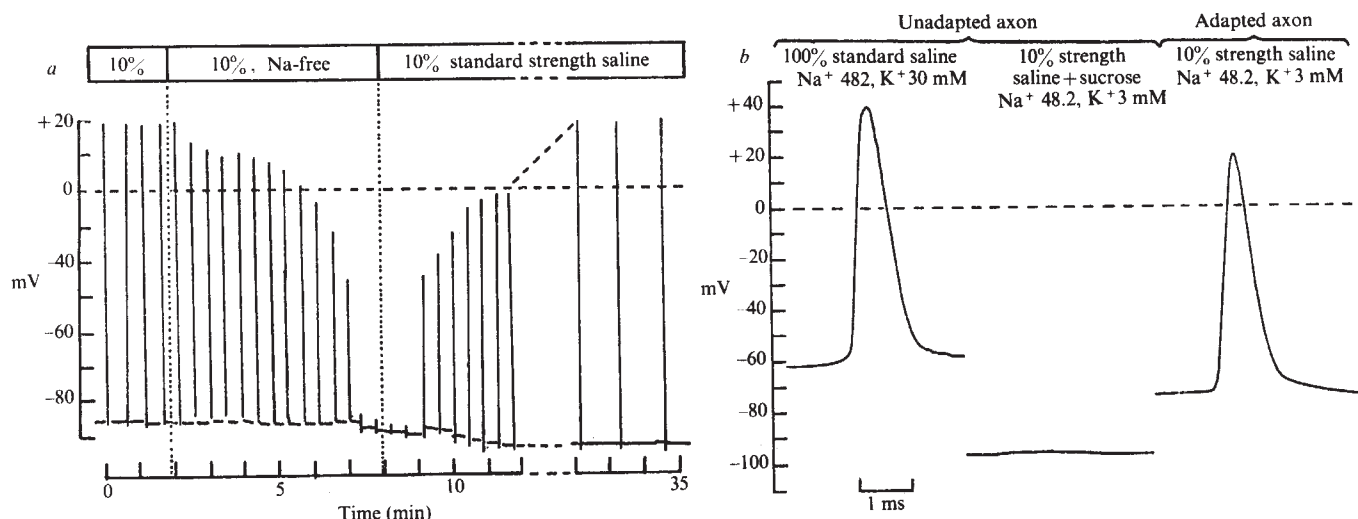


Fig. 4 Axonal action potentials recorded from individuals reared for 10 weeks in flowing distilled water. *a*, The effects of sodium deficiency during exposure to 10% standard strength, hyposmotic, saline. *b*, Action potentials recorded from an unadapted axon, in normal saline, and from a hyposmotically adapted one in 10%, hyposmotic saline. Exposure of the unadapted axon to isosmotic (sucrose-substituted) 10% strength saline caused conduction block and hyperpolarisation of the axon membrane.

by dilute media which would be insufficient to maintain excitability in unadapted nerve cells.

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Isometric contractile properties of single isolated smooth muscle cells

THE use of multicellular strips to study contractile properties of smooth muscle cells rests on the assumption that properties of individual cells are directly reflected in the behaviour of whole tissues. Both the heterogeneity of the contractile state of the cell population in the intact strip, and the complex interconnections between cells and extracellular fibrous elements, however, must influence measured mechanical properties. Methods for isolating viable single smooth muscle fibres from the stomach of the toad, *Bufo marinus*^{1,2} now make it possible to make direct measurements of force generation in single cells. I report here a technique for isometrically measuring the force of con-

traction of a single isolated smooth muscle cell. The method was used to investigate the kinetics and magnitude of force development in a single smooth muscle cell. The results reveal that the maximum force per cm² of a single cell is comparable with that of whole tissue. The onset of active force development after stimulation is exceptionally slow. Analysis of this delay suggests that it resides in step(s), perhaps unique to smooth muscle, whereby Ca²⁺ activates the contractile machinery.

Suspensions of isolated single smooth muscle cells were prepared by enzymatic digestion of the stomach muscularis from *Bufo marinus* by a modification^{2,3} of the procedure of Bagby *et al.*¹. Individual cells were tied in a knot at each end around a probe as shown in Fig. 1*a*. One probe was attached to an ultrasensitive force transducer⁴ able to measure forces as small as a few μ g while the other probe was attached to a micromanipulator. Attachment of a cell to the probes was facilitated by the anionic exchange resin particle at the end of each probe. An electrostatic attraction between the resin particle and the cell facilitates a weak attachment of a probe at each end of the cell. This attachment is strengthened by tying the cell in a knot around each probe using a micromanipulator. The resulting attachment is sufficiently strong to withstand the force of contraction of the cell. When experiments failed they did so quite often because the cell ripped itself in half, rather than pulling free at its attachment. Activation was accomplished by passage of a brief electric current between two Ag-AgCl 'paddles' (350 μ m diameter) placed on either side of the cell.

A typical contractile response to a single submaximal shock is shown in Fig. 1*b*. In this cell a peak contractile force of 179 μ g was achieved in 11.5 s. In 20 cells stimulated in a similar manner the average time to peak force was 11.0 $\pm$ 1.0 (s.e.) s. The mean half-time for relaxation in these cells was 7.7 $\pm$ 0.9 (s.e.) s. Peak contractile force depended on stimulus intensity, as shown in Fig. 1*c*. In isotonic studies of similar isolated cells the extent of shortening also varied as a function of stimulus magnitude^{2,5}. The maximum contractile force recorded from more than 80 single smooth muscle cells was 2.6 kg cm⁻², which is similar to the maximum force measured in intact smooth muscle strips^{6,7}. The maximum force per cm² produced by a single smooth muscle cell is also comparable with the maximum force produced by a single frog skeletal muscle fibre⁸.

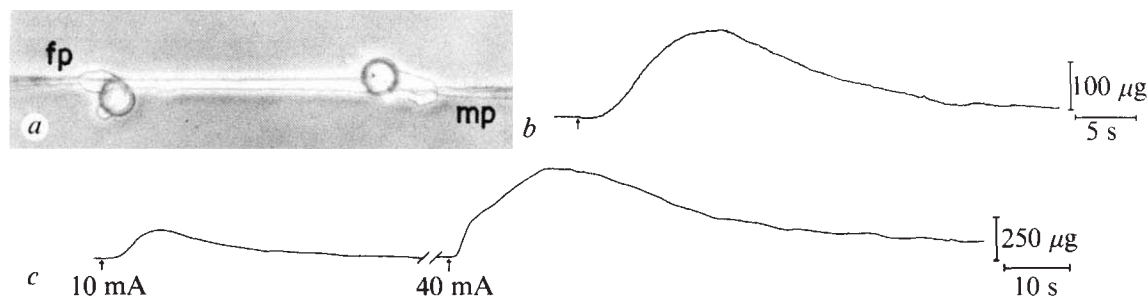


Fig. 1 *a*, Photomicrograph (phase contrast, $\times 390$) of a single smooth muscle cell knotted at each end around a probe to facilitate isometric measurements of force. One probe (fp) was attached to a force transducer whereas the other probe (mp) was attached to a Kopf Hydraulic Microdrive (Model 1207S) or a Burleigh Piezoelectric Translator (Model PZ-30). When using the piezoelectric translator an eddy current sensor (Kaman Sciences, Model KD-2000-.5SU) was used to measure displacement amplitudes and timecourse. The microdrive was used for large amplitude stretches, and the piezoelectric device for small sinusoidal oscillations. Cell length was adjusted so that resting force was between 5 and 12 μg . Experiments were performed at room temperature (19–22 °C). Most cells used were exposed to 10 μM isoproterenol to ensure that they remained relaxed during the knotting procedure. At this concentration isoproterenol has a potent relaxing effect on the isolated cells which can be overcome by electrical or other stimulation³⁶. *b*, Isometric recording of contractile response of a single smooth muscle cell to a submaximal electrical stimulation. Stimulus was a 1-ms square wave of 14-mA amplitude and occurred at the arrow. Maximum force achieved was 179 μg , or 0.41 kg cm^{-2} . Cross-sectional area of individual fibres on which force measurements were made was calculated assuming the cells to have a circular profile. Cell diameter was measured midway along the length of the cell from video recordings through a microscope; the video image gave a $\times 500$ magnification of the cell. Cross sections of fixed single smooth muscle cells revealed a basically circular profile. *c*, Isometric recordings of contractile response of a single smooth muscle cell to two levels of electrical stimulation. The stimulus, a 1-ms square wave whose amplitude is indicated below the records, was initiated at arrow. Maximum forces achieved were 155 μg (0.55 kg cm^{-2}) and 512 μg (1.81 kg cm^{-2}) in response to 10- and 40-mA pulses, respectively.

Considering the much lower myosin content in smooth muscle (20% of that in skeletal muscle⁹) it is remarkable that similar forces can be generated. Although myosin in smooth muscle seems to be organised into slightly longer filaments (2.2 μm) than in striated muscle (1.5–1.6 μm)¹⁰, the increased filament length *per se* can account only to a small extent (27%) for the apparent increase in force per cm^2 per g myosin. An additional explanation may derive from studies of changes in the ultrastructure^{2,11} birefringence¹², and orientation of birefringent 'fibrils' in isolated smooth muscle cells¹³ during isotonic contraction, which suggest that contractile elements are oriented obliquely to the cells' long axis. As Rosenbluth pointed out¹⁴, with this arrangement there are more force-generating elements effectively in parallel within the cell than if the same elements ran end-to-end in series, as in skeletal muscle.

There is a considerable delay between electrical activation of a smooth muscle cell and the onset of active force development. The mean latency between a single 1-ms shock of supramaximal intensity and the development of force was 214 ± 13 ms (mean $\pm$ s.e.; 60 cells). The time course of the onset of force development in a single cell is shown in Fig. 2*a*, where there is a delay of 170 ms be-

tween stimulation and an increase in force. Values for the latency in the 60 cells studied varied from 50 to 500 ms, so that Fig. 2*a* represents one of the shorter latencies. Previously, when preliminary results were presented, even longer latencies were noted, but re-examination of the force recordings containing these very long latencies ($>2,000$ ms) revealed that the cells were not maximally activated, as judged by the very low values for peak dP/dt . The latency in the smooth muscle cells reported here is very much longer than that in skeletal muscle in similar conditions (2 ms in frog skeletal muscle at 20 °C (ref. 16)); it is also longer than the minimum latency observed in amphibian cardiac muscle (15–20 ms in frog ventricle¹⁷). Long delays for development of active force have also been noted in intact strips of smooth muscle. For example, there is a 80–160-ms interval between an action potential and the onset of contraction (measured indirectly) in uterine strips at 30 °C (ref. 18).

Although the rate-limiting step responsible for the long latencies in the isolated smooth muscle cells has not been identified, several potential causes for the long delay can be eliminated. The long latencies cannot be attributed to an effect of isoproterenol (10 μM), present in most experiments because, in the absence of isoproterenol, similarly

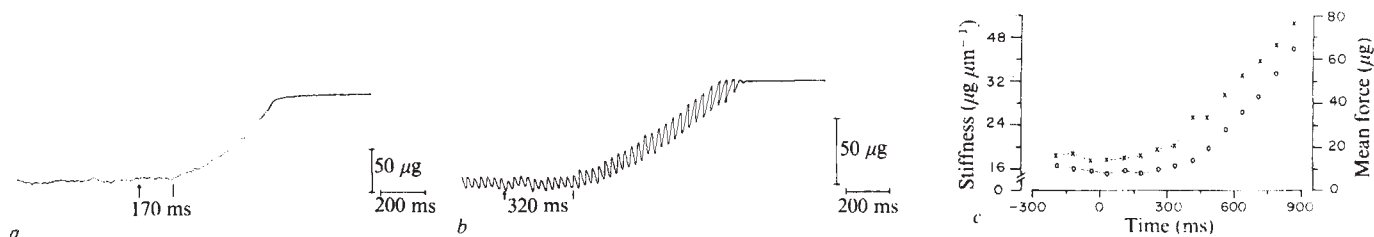


Fig. 2 *a*, Isometric recording of the contractile response of a single smooth muscle cell to supramaximal electrical stimulation. Stimulus (1-ms, 55-mA square wave) was initiated at the arrow on the record. 170 ms elapsed between stimulation and onset of measurable active force. Maximum force developed by this cell was 698 μg (1.1 kg cm^{-2}). Due to the high gain used to facilitate early detection of active force development, amplifier saturates (flat right-hand portion of record) 700 ms after stimulation. *b*, Isometric recording of the contractile response of a single smooth muscle to supramaximal electrical stimulation while undergoing sinusoidal length oscillations. Stimulus (1-ms, 62-mA square wave) was initiated at the arrow. Sinusoidal length oscillations (33 Hz) were 0.5 μm peak to peak. Cell length between knots was 186 μm . Flat portion of the record (right-hand portion of trace) indicates saturation of amplifier at high gain used. Maximum force achieved was 249 μg (0.56 kg cm^{-2}). *c*, Average force (○) and stiffness (×) as a function of time before and after electrical stimulation for the cell in Fig. 2*b*. Stimulation occurred at zero time. Mean force was taken as the midpoint of the force trace in Fig. 2*b*. The plotted values for stiffness represent the mean stiffness during successive 75-ms periods; the latency for the onset of active force development was 320 ms; stiffness did not increase until active force was measurable.

long latencies were noted (254 ± 34 ms; mean $\pm$ s.e.; $n=4$). The delay does not seem to be in the ability of the contractile apparatus, once activated, to produce measurable force. If slack within the contractile system, or a delay between attachment of cross-bridges and the initiation of their cycling were responsible for the long latencies, muscle stiffness, which is associated with cross-bridge attachment in striated muscles¹⁹⁻²³, would probably increase before active force development. This is not the case. When muscle stiffness is measured either by applying small sinusoidal length oscillations ($10-100$ cycles s^{-1} ; $0.5-2$ μ m amplitude) to the muscle or when rapid larger amplitude stretches are performed before and then at various times after stimulation, no increase in stiffness is measured until after active force is produced (Fig. 2b and c; Table 1). Although it might be argued that stiffness in smooth muscle, unlike that in other muscles, is unrelated to cross-bridge attachment, this is unlikely, because a generally linear relation between stiffness and active force development has been noted in individual fibres (Fig. 2b and c). A similar relation between stiffness and active force²⁴ was seen in intact smooth muscle tissues but the unknown contribution of extracellular elastic elements to such measurements makes the interpretation of results on intact tissue uncertain. The

must reside in a step between the stimulus-induced potential change and the attachment of cross-bridges.

On theoretical grounds it is unlikely that the long delay reflects the time required for diffusion of Ca^{2+} from its site of release to the myofibrils. Even if Ca^{2+} had to diffuse from the surface membrane to a myofibril at the central axis of the cell (a 'worst case') diffusional equilibration would be 90% complete within 10 ms (ref. 25). As indicated above, typical latencies are much longer than this. Unlike cardiac muscle^{17,26}, the latency for force development in smooth muscle does not seem to reflect the time required for a slow but continuous release of Ca^{2+} to achieve a threshold required for observable tension development. Such a release mechanism might be slow in an absolute sense or might be effectively slow due to close matching of rates for Ca^{2+} release and re-uptake. If the latency reflected the time for a slow release of Ca^{2+} to achieve a threshold level, then the latency to a suprathreshold stimulus ought to be greatly reduced in a cell already producing some active force, due levels would presumably be above the hypothetical threshold to a preceding submaximal activation. In such cells Ca^{2+} hold when the supramaximal stimulus is applied. As Table 2 shows, there is no significant reduction in the latency to a supramaximal stimulus in cells already pro-

Table 1 Relative resistance to quick stretches applied before and after stimulation

Time between onset of stimulus and completion of quick stretch* (ms)	Resistance to stretch† relative to unstimulated cell	Force just before quick stretch‡
50-100	0.96 ± 0.10 ($n=9$)	0
100-200	0.94 ± 0.11 ($n=8$)	0
1,060	3.21 ± 0.49 ($n=3$)	$0.12 P_{max}$

*Stretches applied as a ramp at 1 μ m per 6.2 ms. Stretch amplitude averaged 8 μ m; normalised for cell length this corresponds to an average of 5.3% of cell length just before the quick stretch.

†Resistance to stretch was taken as the peak transient increase in force resulting from the sudden length increment. Values are the mean $\pm$ s.e. of the ratio of the resistance to identical length increments applied several seconds before and then at the time noted after stimulation. The number of cells is indicated in parentheses.

‡Active force existing in the fibre at the initiation of quick stretch relative to the maximum measured force. Value is the mean for the number of the number of cells used in resistance determination.

possibility that the latency reflects the time required to eliminate slack within the contractile system is further rendered unlikely by the observation that a rapid stretch of 9-11 μ m (accomplished as a ramp in 60 ms) given 40 ms after the stimulus did not reduce the mean latency (285 ± 20 ms; $n=7$) below that of a control group (249 ± 33 ms; $n=7$). These results indicate that the latency

producing active force due to a shortly preceding submaximal stimulus. A further argument against the long delays reflecting the time required to achieve a threshold level of Ca^{2+} rests on the finding that the latency was not affected either by raising Ca^{2+} in the medium from 1.8 to 20 mM or by eliminating Ca^{2+} from the medium.

These observations on the latency of force generation suggest either that the long delay resides in the coupling of transmembrane potential changes to the opening of a Ca^{2+} gate or that there is a considerable delay between the arrival of Ca^{2+} at the contractile proteins and the formation of cross-bridges between myosin and actin. A possible explanation for the long delays is suggested by a recently reported^{27,28} difference in the mode of activation of vertebrate smooth and striated muscle. In smooth muscle it has been proposed^{29,30} that Ca^{2+} activation is accomplished by phosphorylation of a myosin light chain, and the delays could be imposed by the phosphorylation. In striated muscles where the latency is much shorter, activation seems merely to involve Ca^{2+} binding to troponin followed by a slight rotation of the troponin-tropomyosin complex³¹⁻³⁵. Continued studies of the mechanical properties of isolated single smooth muscle cells may aid in further understanding both the activation process and the mechanism of force generation in smooth muscle.

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Table 2 Effect of a preceding submaximal activation on the latency between supramaximal electrical stimulation and the onset of active force

Conditions	Latency (ms $\pm$ s.e.)
Supramaximal stimulus	201 ± 42 ($n=11$)
Supramaximal stimulus, applied during submaximal contraction*	166 ± 26 ($n=8$)

Latencies were determined after a supramaximal stimulus (1-ms, 50-60-mA square wave) for the number of cells in parentheses. Only latency data from those suspensions where the effect of a supramaximal stimulus alone and the effect of a supramaximal stimulus applied after a submaximal stimulus was tested have been included in the mean latencies.

*Submaximal contraction was initiated by a 10-15-mA stimulus, 1-ms square wave; supramaximal stimulus was applied during the plateau of the submaximal contraction.

discussions with Drs T. Honeyman, J. J. Singer and J. V. Walsh.

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Increased optical transparency associated with excitation-contraction coupling in voltage-clamped cut skeletal muscle fibres

CONTRACTION of a skeletal muscle fibre is triggered by depolarisation of its transverse (T)-tubules¹, which occurs physiologically with the spread of an action potential into the T-system². The T-tubule depolarisation gives rise to calcium release^{3,4} from the adjacent but separate^{5,6} sarcoplasmic reticulum (SR). The released calcium binds to troponin, removing the inhibition of actin-myosin interaction and enabling contractile activity⁷. Little is known about the nature of the T-tubule-SR interaction. It has been suggested that movement of charged molecules or dipoles in the T-tubule membrane is the initial voltage-sensitive step in the chain of events leading from T-tubule depolarisation to SR calcium release⁸, and membrane charge displacement currents which may reflect such movements have been observed and characterised^{9–10}. We report here a study of subsequent changes which may be related to calcium release.

Whole muscles or muscle fibre bundles stimulated to produce propagated action potentials exhibit an increase in optical transparency shortly after the action potential^{11,12}. In these conditions the transparency increase follows a time course generally similar to that of latency relaxation¹¹, the fall in muscle tension which precedes the much larger tension development with contraction¹³. Much of the observed difference in transparency and relaxation time courses may be attributable to distortion of the latter due to asynchronous mechanical activation by a propagated action potential¹³. Latency relaxation has been explained on the basis of a mechanical change in the SR accompanying calcium release^{13,14}, so the transparency increase may similarly reflect SR changes associated with calcium release. We have investigated the time- and voltage-dependence of the transparency increase using voltage-clamped cut muscle fibre seg-

ments prepared so as to retain their contractile ability on electrical depolarisation.

With a high-K, Ca-free relaxing solution (120 mM K glutamate, 2 mM MgCl₂, 0.01 mM EGTA, 5 mM Tris-maleate buffer), single semitendinosus or ileofibularis muscle fibres from room temperature-adapted summer frogs (*Rana pipiens*) were cut about 0.5–1 cm from the tendon and mounted in a two pool leucite chamber. A single vaseline gap about 0.5–0.6 mm wide separated the pool containing the closed intact fibre segment from the pool containing the cut end. After mounting the fibre, the pools were changed from relaxing solution to the experimental solutions and experiments run at 3.4–4.6 °C.

Both the voltage difference and total current flowing between the pools were monitored and used with an analogue amplifier compensating circuit to produce a voltage V_m' equal to the fibre membrane potential just beyond the gap in the closed end pool. The compensating circuit corrected for voltage drops along the internal resistance of the length of fibre in the gap in a manner similar to standard 'compensated feedback'¹⁵ and also corrected for leakage current across the gap outside the fibre (our work in preparation). A microelectrode was inserted in the closed fibre segment just beyond the gap in order to set the compensating circuit, but the electrode could be removed after setting the circuit. Thereafter, a voltage clamp circuit was used to maintain V_m' at a holding potential of –100 mV and to produce voltage pulses from the holding potential. Using cut fibres isolated from cold-adapted frogs, strength-duration curves for just detectable movement on depolarisation at 3–4 °C (ref. 16) closely resembled those reported for intact fibres under similar conditions¹⁷. Cut fibres from room temperature-adapted frogs required larger depolarisations for contraction (our unpublished work).

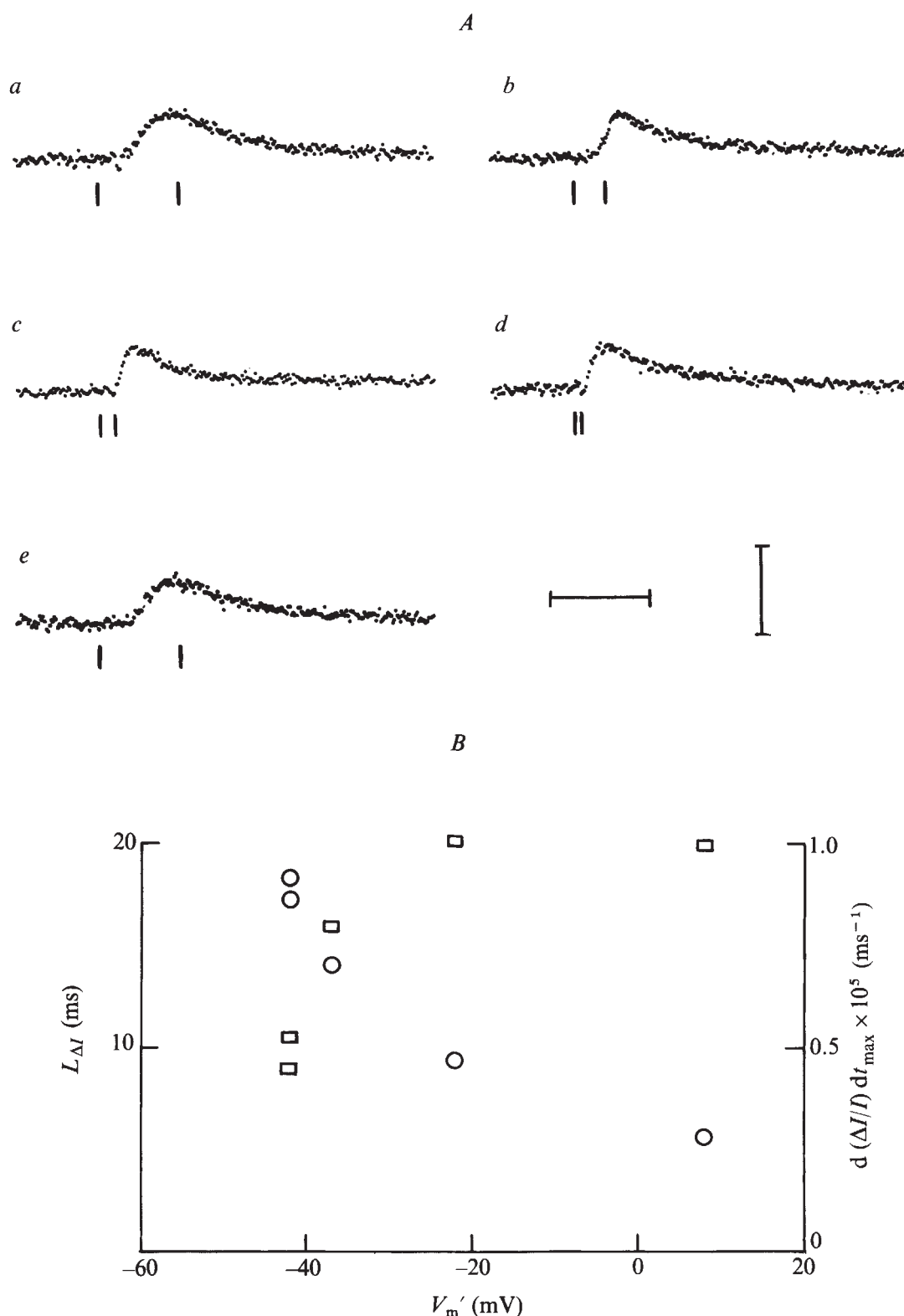
A reduced size image of a uniformly illuminated slit was focused on the fibre and the slit width adjusted so that the slit image, which was in all cases parallel to the fibre and 170 µm long, occupied the middle third to half of the fibre width. After positioning the fibre and focusing and adjusting the slit under the light microscope, a prism in the microscope was moved out of the light path. This focused the image of fibre and slit on a photodiode used for monitoring the transmitted light intensity. Measured changes ΔI in transmitted light intensity were divided by the light intensity I transmitted by the resting fibre.

In all experiments care was taken to keep the depolarisations of a given duration below the level which produced microscopically visible movement. $\Delta I/I$ records for larger pulses which did produce movement exhibited a second component, a decrease in transparency, which occurred during the falling phase of the transparency increases described below. This component was not characterised.

Figure 1A presents $\Delta I/I$ records for pulses of four different durations, with the amplitude of each pulse preselected on the basis of being within a few mV of the threshold for visible movement at that duration. The peak value of $\Delta I/I$ in each case was approximately the same. The $\Delta I/I$ time courses, however, differed considerably. Increasing the size of the depolarisation decreased the ΔI latency and increased the maximum rate of ΔI increase following the latent period. A repeat run using the first pulse (record 1Ae) shows that drift in the preparation with time was negligible. The time courses of all records in Fig. 1A are too slow for the transparency increase to be originating directly from membrane potential changes across surface¹⁸ or T-tubule¹⁹ membranes. It probably originates from either an SR change accompanying calcium release or from a subsequent contractile filament change preceding mechanical activity. Considering that different amplitude and duration pulses which were all close to threshold for mechanical activity were also all accompanied by about the same peak increase in transparency, the $\Delta I/I$ amplitude seems to be related to either the amount of calcium released from the SR or to the degree of change in the myofilaments.

Figure 1B shows the relationship between V_m' during the pulse and the latency $L_{\Delta I}$ and maximum rate of rise $d(\Delta I/I)/dt_{\max}$ of

Fig. 1 Transparency changes due to pulses close to threshold for initiating microscopically visible movement. **A**, $\Delta I/I$ records for pulses to the following V_m' values (mV) for the durations (ms) indicated in parentheses. *a*, -42 (50); *b*, -37 (20); *c*, -22 (10); *d*, +8 (5); *e*, -42 (50). Each record gives the average of 10 individual traces. The horizontal calibration bar represents 60 ms, and the vertical 2×10^{-4} . Here and in Figs 2 and 3A the vertical lines below each trace mark the first and last interval during the pulse. **B**, Latency ($L_{\Delta I}$) in the onset of the transparency increase ($\circ$) and maximum rate of transparency increase $d(\Delta I/I)/dt_{\max}$ ($\square$) as a function of membrane potential during the pulse for the traces in part A. The parameters were determined from a least squares fit of a straight line through the apparently linearly rising phase of $\Delta I/I$. The closed (tendon) end pool contained 75 mM (TEA)₂SO₄, 5 mM Cs₂SO₄, 7.4 mM total CaSO₄, 5 mM Tris-maleate buffer, and tetrodotoxin (TTX) at 10^{-7} g ml⁻¹. The open end pool contained 120 mM Cs glutamate, 2 mM MgCl₂, 0.5 mM ATP, 1 mM EGTA and 5 mM Tris-maleate buffer (applied 70 min before start of this run). These and all other solutions had pH values of 7.0 ± 0.1 at room temperature. Sarcomere length $S = 3.01$ μ m, length l_c of intact fibre in the closed end pool = 500 μ m, fibre width d in the plane perpendicular to the microscope axis = 64 μ m. Fibre 68, 3.8 °C. Fibres were illuminated using a battery-powered tungsten-halogen lamp, condensing lenses, a slit of adjustable width, a 525-nm central wave length 25-nm half-peak bandwidth interference filter (Baird Atomic 35-14-7), a microscope objective (10 $\times$, 0.25 NA: Nikon 77795) as final condenser, and a glass cover slip as the chamber floor and were observed using a compound microscope with a water immersion objective (40 $\times$, 0.75 NA: Zeiss 56 17 02) and 10 $\times$ wide-field eye pieces (Nikon). A photodiode (Electro-Nuclear PDS-050GB) connected to a current measuring operational amplifier circuit was used to monitor transmitted light intensity I . Intensity changes ΔI were monitored by using an a.c. coupled high-gain amplifier (Tektronix 3A9, 1 kHz bandwidth) in series with the photodiode amplifier. A voltage-to-frequency converter (Anadex 1700-5044-00) and digital counters were used to digitise the average output voltage of the 3A9 amplifier for successive 1-ms intervals⁹ and a PDP-computer (Digital Equipment Co.) was used to store and signal average the ΔI records. I values of resting fibres were monitored by using a d.c. coupled digital voltmeter (Fluke 8000A).



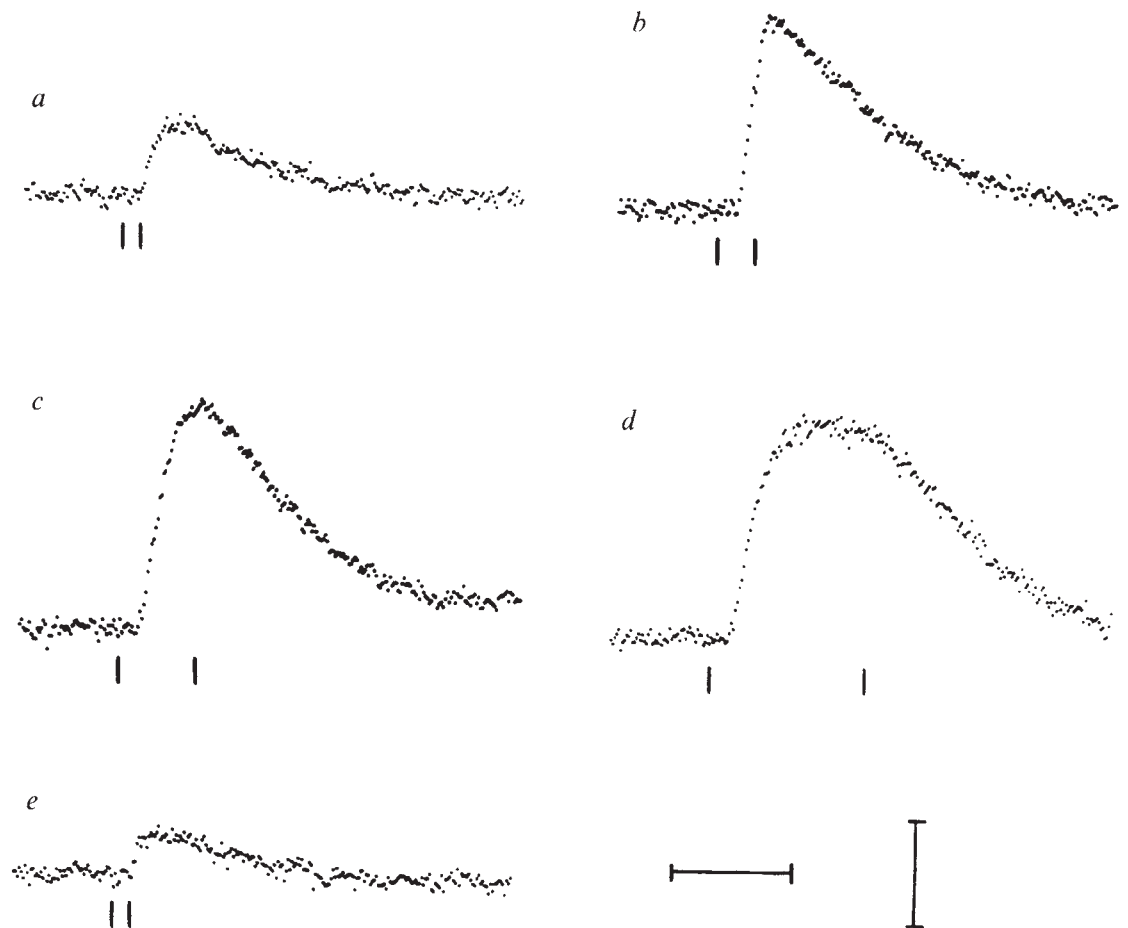


Fig. 2 Effect of pulse duration on the transparency increase. V_m' during all pulses = -14 mV. Pulse durations (ms): *a*, 10; *b*, 20; *c*, 40; *d*, 80; *e*, 10. Each record is an average of five individual traces. Before and after this run, small fibre movements were observed by using the 40- and 80-ms pulses, but not with the 10- and 20-ms pulses. The horizontal calibration bar represents 60 ms, vertical 2×10^{-4} . Solutions used were as in Fig. 1 (applied 200 min before start of this run). $S = 2.81 \mu\text{m}$, $l_c = 510 \mu\text{m}$, $d = 73 \mu\text{m}$, Fibre 69, 4.0°C .

$\Delta I/I$ for the records in Fig. 1*A*. The minimum latency of 6–10 ms observed in this and other fibres for pulses to V_m' values slightly positive to 0 mV is consistent with the values of 6.5–7.9 ms for the time to onset of latency relaxation in massively stimulated frog sartorius muscles at 0°C (ref. 20). Latencies such as those in Fig. 1*B* cannot be accounted for on the basis of delays in membrane charge movement^{9,10}. If the transparency increase originates from the SR, the latencies suggest that there must be either one or more intermediate steps between charge movement and the SR change or that a number of charges must move before the SR change can occur. If it originates from myofilament changes, the latencies would be made up of both the response time of the SR and the time for released calcium to reach and react with the myofilaments.

In many cases the final decay of $\Delta I/I$ was exponential, with time constants of 33–85 ms in 6 fibres. With other records, a better fit was obtained using an exponential decay to a new baseline level, perhaps indicating the presence of a small amount of another ΔI component in these records.

Figure 2 presents records from another fibre for a run in which pulses of the same amplitude but different durations were used. Increasing the pulse duration from 10 to 20 ms caused a large increase in the maximum $\Delta I/I$ (records *a* and *b*), whereas further lengthening the pulse to 40 ms (*c*) caused only a slight further increase in $\Delta I/I$. Increasing the pulse duration from 40 to 80 ms (*c* and *d*) caused no further increase in amplitude but introduced a plateau phase into the $\Delta I/I$ record.

A third type of experiment involved pulses of constant duration but varying amplitude. Figure 3*A* presents records from this type of experiment carried out using 60-ms pulses with a fibre in Ringer solution which showed no movement for

relatively large and long pulses. In this case the signal-to-noise ratio of the $\Delta I/I$ records was sufficiently large that signal averaging was unnecessary and single sweeps could be used. If present, any changes in I for negative pulses to V_m' values of up to -166 mV (record *a*) or for positive pulses to V_m' values down to -44 mV (record *b*) were obscured by baseline noise. Larger depolarisations caused transparency changes which increased with the size of the depolarisation. The variation of peak $\Delta I/I$ with pulse V_m' for the records in Fig. 3*A* is shown in Fig. 3*B*. These peak values represent the maximum $\Delta I/I$ attainable at the given V_m' in this fibre since in each case the pulse ended after the peak in $\Delta I/I$ had been attained. Pulses to voltages slightly positive to 0 mV seemed to have produced a maximal increase in transparency.

The transparency increase which we have studied may originate from either SR or myofilament changes preceding and related to mechanical activity. It does not seem to be a movement artefact, however, because pulses causing no detectable movement can produce transparency increases, and pulses which cause movement also produce a later component of decreased transparency which coincides in time with the movement (our unpublished observation). If the transparency increase is due to myofilament changes, the similarity of its time course to that of latency relaxation would support the hypothesis that latency relaxation also originates in the myofilaments²¹ and not in the SR. Independently of the origin of the ΔI signals, the manner in which $d(\Delta I/I)/dt_{\text{max}}$ and peak $\Delta I/I$ increase and $L_{\Delta I}$ decreases with increasing depolarisation seems generally consistent with the voltage dependence of membrane charge movement.

The transparency increase time courses reported here resemble the time courses of fluorescence changes seen in intact

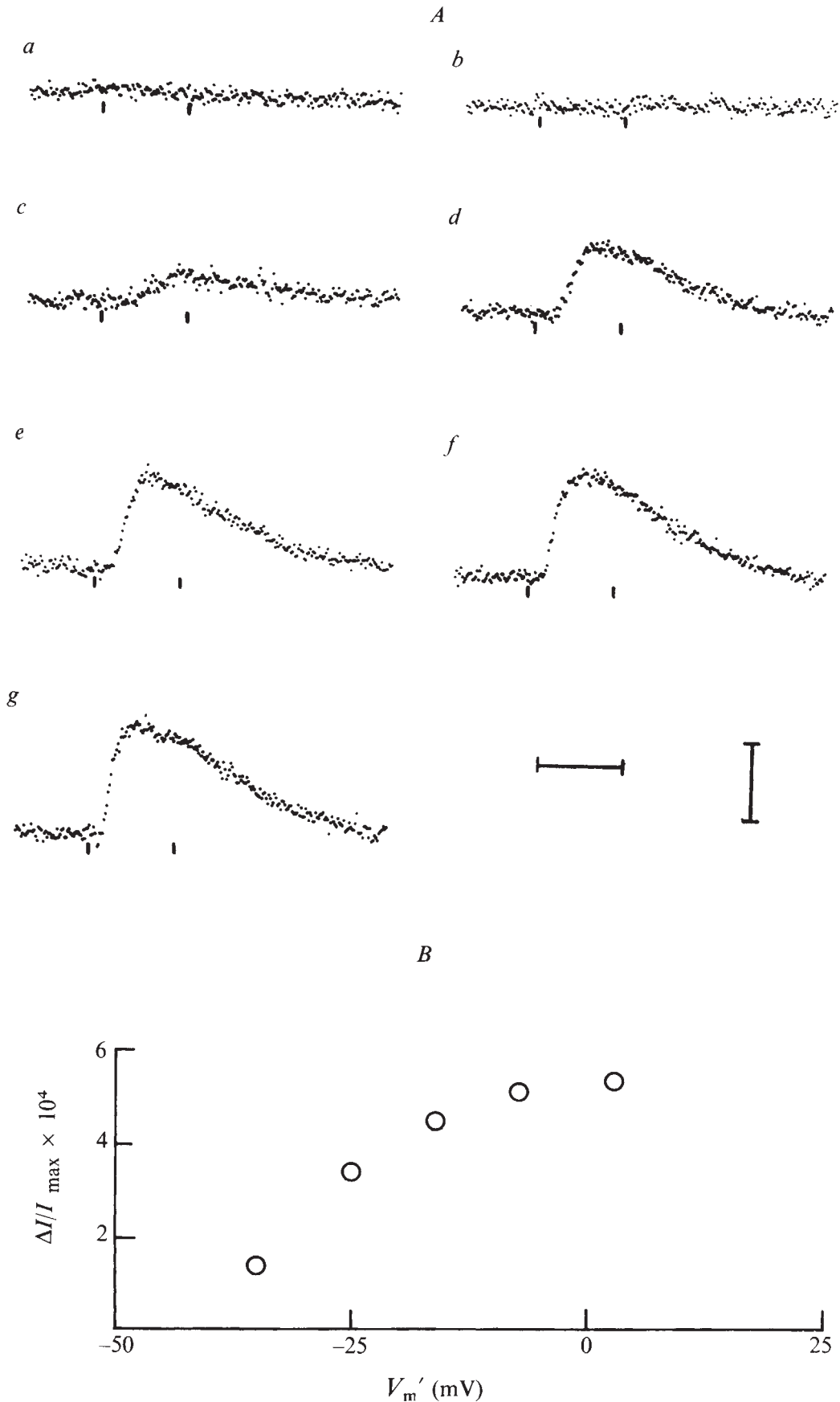


Fig. 3 Effect of pulse amplitude on the transparency increase. *A*, $\Delta I/I$ records for 60-ms pulses to the following V_m' values (mV): *a*, -165; *b*, -44; *c*, -35; *d*, -25; *e*, -16; *f*, -7; *g*, +3. Each record represents a single trace, without signal averaging. The horizontal calibration bar represents 60 ms, vertical 4×10^{-4} . *B*, Maximum $\Delta I/I$ as a function of membrane potential during the pulse for the records in (*A*). The closed end pool contained Ringer's solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl_2 , 1 mM Tris-maleate buffer) with TTX at 10^{-7} g ml $^{-1}$. The open end solution was identical to that in Figs 1 and 2 except that K glutamate (120 mM) was used in place of Cs glutamate (applied 65 min before the start of this run). $S = 2.69 \mu\text{m}$, $l_c = 500 \mu\text{m}$, $d = 84 \mu\text{m}$. Fibre 64, 3.9 °C.

muscles stained with a penetrating membrane potential-sensitive fluorescent dye and depolarised by current steps applied through fluid electrodes²², although exact comparison is not possible because of differences in experimental conditions. Since intact single muscle fibres exposed to fluorescent dyes exhibit fluorescence and birefringence changes having similar time courses in response to action potentials²³, it seems likely that the fluorescence changes observed using penetrating dyes^{22,23}, the second component birefringence change²⁴ and the transparency increase reported here may all monitor the same events.

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Transfer of virulence *in vivo* and *in vitro* in *Agrobacterium*

TRANSFER of virulence from a pathogenic to a non-pathogenic strain of *Agrobacterium* has been reported to occur *in vivo*^{1,2} and is due to the transfer of a plasmid which codes for virulence^{3,4}. In the experimental conditions described, virulence transfer can be detected only after several weeks. The reason for this delay is not clear. The most likely explanations are (1) that infrequent transfer occurs long before it can be detected, detection being dependent on a subsequent increase in the ratio of converted to unconverted recipients or (2) that conditions suitable for virulence transfer occur only after donor and recipient have been in contact for several weeks. The main difficulty in distinguishing between these two alternatives is technical. When pathogenicity testing is used to detect virulence transfer, low frequency transfer is impossible to detect unless thousands of inoculations are made at each assessment. It is known, however, that the virulence plasmid codes for characters other than virulence⁵⁻⁷. One of these is utilisation of octopine and, as Kerr⁸ has pointed out, this could be used instead of pathogenicity testing to detect plasmid transfer. This communication describes both *in vivo* and *in vitro* transfer of virulence using octopine utilisation to detect plasmid transfer.

An experiment was designed to try to detect low frequency transfer of virulence *in vivo*. Strain NCPPB 1001 was used as a donor; it is pathogenic and utilises octopine but is sensitive to rifampicin (25 µg ml⁻¹) and to streptomycin (500 µg ml⁻¹). Double mutation to antibiotic resistance occurs at a frequency of less than 2×10^{-10} . Strain K57 *rif-r str-r* was used as a recipient; it is non-pathogenic, unable to utilise octopine but is resistant to rifampicin and streptomycin. Suspensions of donor and recipient from 48-h cultures on yeast mannitol agar were prepared and mixed in the ratio of 10:1. There were 6×10^8 donor cells per ml. Separate suspensions of donor and recipient were also prepared. These three suspensions were used separately to inoculate tomato seedlings using 15 replicates per treatment. Each week for 5 weeks, three plants from each treatment were removed, the inoculated tissue cut out and macerated separately in 10 ml of sterile distilled water. The macerate (0.1 ml), both undiluted and diluted 10^{-3} , was transferred to agar plates and spread with an L-shaped glass rod. The basal medium contained, per l of water: K₂HPO₄, 10.5 g; KH₂PO₄, 4.5 g; MgSO₄·7H₂O, 0.2 g; CaCl₂, 10 mg; FeSO₄, 5 mg; MnCl₂, 2 mg; and agar (separately sterilised), 20 g. Separately sterilised octopine, 2 g; streptomycin, 0.5 g; actidione, 0.25 g and rifampicin, 25 mg per l were added just before the plates were poured. Actidione was added to prevent fungal contamination. Plasmid transconjugants which combine octopine utilisation of the donor and antibiotic resistance of the recipient grow well on this medium and can easily be distinguished from the 'ghost' colonies of the unconverted recipient. The donor strain is eliminated because it is sensitive to the antibiotics streptomycin and rifampicin. No transconjugant was detected (frequency $< 10^{-5}$) 1 or 2 weeks after inoculation. Transconjugants were detected after 3, 4 and 5 weeks at a frequency per recipient of 4.0×10^{-4} , 4.8×10^{-3} and 3.7×10^{-2} respectively. The increase in the frequency of transconjugants with time is significant ($P = 0.011$ by the Kruskal Wallis one-way analysis of variance on ranks).

These results support the hypothesis that infrequent plasmid transfer occurs followed by an increase in the ratio of converted to unconverted recipients. This might be explained by the presence of octopine in galls induced by strain NCPPB 1001 (ref. 7). Converted recipients can utilise octopine and this might give them a biological advantage over unconverted recipients and explain the increase with time in the ratio of these two forms. If correct, perhaps transfer of virulence *in vitro* could be detected by exposing a mixture of donor and recipient to octopine.

We attempted to transfer virulence *in vitro*. Suspensions of donor and recipient were prepared as in the previous experiment. The bacteria in suspension were then deposited on Millipore filters (pore size 0.22 µm) which were then placed on the surface of the basal medium to which octopine (2 g l⁻¹) and actidione (0.25 g l⁻¹) had been added. Two filters from each treatment (donor alone, recipient alone and a mixture of donor and recipient at a ratio of 10:1) were transferred separately after 1, 2, 4 and 7 d to 10 ml of sterile water, the bacteria suspended and plated on the selective medium containing antibiotics. The donor was eliminated: the recipient by itself gave only 'ghost' colonies. The mixture of donor and recipient yielded transconjugants at each assessment, their frequency being 1.4×10^{-2} , 1.9×10^{-2} , 7.1×10^{-2} , and 2.5×10^{-1} after 1, 2, 4 and 7 d, respectively. Again there was a significant increase in transconjugant frequency with time ($P < 0.025$).

If transconjugants are detected because they are favoured by the presence of octopine, removal of octopine should drastically reduce their frequency. An experiment was designed to test this. Bacteria were deposited on filters, as in the previous experiment, but the filters were placed on three different media: (1) basal medium plus octopine plus actidione, (2) basal medium plus actidione and (3) basal medium plus arginine (2 g l⁻¹) plus actidione. Both donor and recipient can utilise arginine as a source of both carbon and nitrogen, whereas octopine (N²-(D-1-carboxyethyl)-L-arginine) can be utilised as growth substrate by the donor only. The filters were removed after 3 and 7 d, the bacteria suspended in water and plated on the selective medium. Transconjugants were detected only when donor and recipient were exposed to octopine, their frequency being

2.2×10^{-5} after 3 d and 2.5×10^{-1} after 7 d. When octopine was omitted or replaced with arginine, frequency of transconjugants was less than 5.0×10^{-8} after 3 d and less than 1.5×10^{-8} after 7 d. These results further support the hypothesis that transconjugants are favoured by the presence of octopine. A possible interpretation is that octopine promotes plasmid transfer.

The octopine-utilising strains A6, B6 and NCPPB 4 were also used as donors with strain K57 *rif-r str-r* as recipient. After 5 d exposure to octopine in the basal medium recombinants were obtained at a mean frequency of 7×10^{-2} . Presumably the plasmid from nopaline-utilising strains could also be transferred and detected *in vitro* if nopaline, instead of octopine, were added to the basal medium. Unfortunately, nopaline is not commercially available and this restricts its use. But mutants of nopaline strains, constitutive for nopaline metabolism, can be obtained by plating a large number of cells on a medium containing octopine as the sole source of carbon and nitrogen⁹; these mutants can utilise octopine as well as nopaline. Three such mutants of strains C-58, K14 and K24 were selected and used as donors with K57 *rif-r str-r* as recipient using the conditions described above for successful transfer of virulence *in vitro*. Transconjugants were obtained at a mean frequency of 2×10^{-4} . This result indicates that the mutation to constitutivity is borne on the plasmid.

There is still a large group of pathogenic *Agrobacterium* which have to be considered. These are biotype 2 pathogens¹⁰. They can utilise both octopine and nopaline but only nopaline utilisation is coded for by the virulence plasmid^{4,7}. It is therefore impossible to select constitutive mutants for nopaline utilisation by plating these strains on octopine. Nopaline metabolism, however, is inducible; after it has been induced, nopaline strains can degrade octopine⁹. This characteristic was used to obtain transfer of virulence *in vitro* using the biotype 2 pathogens TT 133 and K27 as donors and K57 *rif-r str-r* as recipient. The same procedure as above was adopted except that nopaline ($10 \mu\text{g ml}^{-1}$), extracted from gall tissue⁷, was incorporated into both media, in addition to the other constituents. This level of nopaline was sufficient to induce octopine utilisation. Transconjugants at a mean frequency of 4×10^{-6} were obtained. Although this frequency is much lower than that obtained with other donor strains, it is too high to be explained by mutation of wild-type donors to antibiotic resistance. It seems that most pathogenic strains of *Agrobacterium* can be used as donors to transfer the virulence plasmid to an efficient recipient *in vitro*.

In all the experiments described here, presumed plasmid transfer was detected by selecting recombinants which utilised octopine. At every assessment, 20 recombinants and 20 unconverted recipients were subcultured, purified and tested for pathogenicity. All transconjugants were pathogenic; no unconverted recipient was pathogenic.

In vitro transfer of the virulence plasmid of *Agrobacterium* has been achieved recently by other workers who used the fertility plasmid RP4 to promote transfer^{5,6}, but this and the following communication¹¹ are the first reports of plasmid transfer *in vitro* using wild-type donors or their spontaneous mutants.

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Ti plasmids of *Agrobacterium* as conjugative plasmids

THE relationship of the plant-oncogenic properties of *Agrobacterium tumefaciens* to the presence in these strains of large plasmids (the Ti plasmids) has now been convincingly demonstrated in several ways: oncogenic strains cured of the Ti plasmid lose their oncogenicity irreversibly^{1–3}; transfer of Ti plasmids to non-oncogenic plasmidless strains confers oncogenicity to these strains^{3–7}. The first method used to transfer plasmids was based on observations made by Kerr^{8,9}, who had developed a method for the transfer of oncogenicity before the association of oncogenicity with large plasmids was known. It was later demonstrated that this transfer of oncogenicity was due to the transfer of the Ti plasmid^{3,4}. These observations suggested that in certain circumstances the Ti plasmids could behave as conjugative plasmids in promoting bacterial conjugation and plasmid transfer. This paper and its companion¹¹ describe experiments confirming that suggestion, making use of the fact that the capacity of *Agrobacterium* strains to utilise the unusual amino acids octopine (N^2 -(D-1-carboxyethyl)-L-arginine) or nopaline (N^2 -(1, 3-dicarboxypropyl)-L-arginine) is coded for by Ti plasmid linked genes^{3,5–7,11}, and indicate that octopine and nopaline may act in inducing plasmid transfer.

Conjugation experiments were performed with donor and recipient bacteria grown on YEB medium in a shaking waterbath at 28 °C for 12 h. The cells were collected and resuspended in λ -buffer. A mixture of about 10^4 donors and 10^8 recipients was filtered on Millipore filters (pore size 0.45 μm , diameter 25 mm) and the filters were incubated for various periods on solid media consisting either of YEB or octopine or nopaline agar. Filters were then removed and the cells were suspended in λ buffer. After dilution in λ buffer the cells were plated on selective agar media. Filters containing donor or recipient bacteria alone were also prepared and treated similarly.

In a first cross strain ACH-5 (ref. 12) was used as a donor: it is oncogenic, contains a single plasmid 60.6 μm long, utilises octopine but not nopaline as its sole N source and induces tumours which contain octopine but not nopaline. As a recipient we used strain C58-C1, *rif-r*, *ery-r*. This is a heat cured, non-oncogenic, plasmidless derivative of the oncogenic nopaline strain C58 (ref. 4). It was made resistant to $100 \mu\text{g ml}^{-1}$ rifampicin and $150 \mu\text{g ml}^{-1}$ erythromycin by two separate spontaneous mutations. To eliminate the donor cells and select for octopine-utilising recipients the mixture from the filters was plated on the basal medium supplemented with 0.5% glucose, $100 \mu\text{g ml}^{-1}$ octopine, $100 \mu\text{g ml}^{-1}$ rifampicin and $150 \mu\text{g ml}^{-1}$ erythromycin. On this medium the donor cells alone did not form any colonies, whereas the recipient strain formed only 'ghost' colonies. When the filters with the mixture were incubated for 1, 4 and 7 d on YEB agar, no octopine-utilising recipients were detected, indicating that the frequency of such transconjugants was less than 1×10^{-8} in these circumstances. When the filters containing the same mixture were incubated for the same lengths of time on octopine (2 mg ml^{-1}) agar, octopine-utilising colonies were detected with frequencies per recipient bacterium of 5×10^{-4} , 1.5×10^{-3} and 6.4×10^{-3} , respectively. The results summarised in Table 1 demonstrate that all

Table 1 Properties of donor, recipient and transconjugant

Strain	Resistance to 100 µg ml ⁻¹ rifampycin	Resistance to 150 µg ml ⁻¹ erythromycin	Sensitivity to virulent phages of typing set						Ability to utilise octo- or nopaline	Exclusion of phage API	Oncogenicity on pea seedlings	Sensitivity to agrocin 84	Contour length of plasmid (µm)	Fingerprint of <i>EcoRI</i> digest of plasmid
Donors			GS 1	GS 2	GS 3	GS 6	GS 18							
ACH-5	—	—	+	+	—	—	—	+	—	+	+	—	60.6 ± 0.8	ACH5 type
B6S3	—	—	—	—	+	—	—	+	—	+	+	—	(a) 59.0 ± 1.2	(a) ACH5 type
C58	—	—	—	—	—	+	—	—	—	+	+	+	(b) 53.8 ± 0.9	(b) x type
													61.8 ± 1.1	C58 type
Recipient														
C58-C1 <i>rif-r ery-r</i>	+	+	—	+	—	+	—	—	—	—	—	—	no plasmid	
Transconjugants														
C58-C1 (Ti-ACH5)	+	+	—	+	—	+	—	+	—	—	—	—	60.4 ± 1.2	ACH5 type
C58-C1 (Ti-B6S3)	+	+	—	+	—	+	—	+	—	—	—	—	59.2 ± 1.2	ACH5 type
C58-C1 <i>oct-u</i> (B6S3)	+	+	—	+	—	+	—	+	—	—	—	—	no plasmid	
C58-C1 (Ti-C58)	+	+	—	+	—	+	—	—	+	+	+	+	61.8 ± 1.1	C58 type

Resistance to antibiotics was scored as growth on YEB medium containing the antibiotics. Sensitivity to the virulent phages of a typing set was scored as lysis or absence of lysis after spotting of about 10⁷ phages ml⁻¹ on lawns of the tested strains in soft agar. The ability to utilise octopine or nopaline was scored by testing whether the tested strains could or could not form full colonies on octopine or nopaline agar. Exclusion of phage API was scored as the absence of plaque formation by this phage on lawns of the tested strains. Oncogenicity was tested on sterile pea seedlings¹⁴. Plasmids were isolated as supercoiled circular DNA molecules by ultracentrifugation¹⁴ and their contour lengths were measured by electron microscopy¹⁷. Agrocin 84 sensitivity was assayed as described previously¹⁸. Restriction endonuclease digest fingerprints were obtained after digestion of the purified plasmid DNA with the *EcoRI* endonuclease¹⁹ and separation of the fragments on Agarose slab gels²⁰. YEB medium: 0.5% Bacto-Beef extract, 0.1% Bacto-Yeast extract, 0.5% peptone, 0.5% sucrose and 0.002 M MgSO₄, pH adjusted to 7.2. λ buffer: 0.001 M Tris-hydrochloride (pH 7.2), 0.01 M MgSO₄. Basal medium: 0.05 M K₂HPO₄, 0.05 M KH₂PO₄, 0.05 M MgSO₄ · 7H₂O, 2.5 × 10⁻⁴ M NaCl, 10⁻⁴ M CaCl₂ adjusted to pH 7.2 and 1.5% (w/v) agar (Difco). Octopine and nopaline agar: 2 mg ml⁻¹ of sterile octopine or nopaline separately sterilised and added to the basal medium before pouring plates.

the selected transconjugant colonies (C58-C1 (Ti-ACH5)) were indeed recipient cells and had acquired the Ti plasmid from the donor strain.

In a second cross, strain B6S3 (ref. 13) was used as a donor; this strain is oncogenic, contains two different plasmids with respective contour lengths of 59.0 ± 1.2 µm and 53.8 ± 0.9 µm. It utilises octopine but not nopaline as sole N source and tumours induced with this strain contain octopine but not nopaline. As a recipient we again used strain C58-C1 *rif-r, ery-r*. The cross was performed as described above. When the filters with the mixture of donors and recipients were incubated for 1, 4 and 7 d on the octopine (2 mg ml⁻¹) medium, octopine-utilising recipients were detected with frequencies of 2 × 10⁻⁶, 1 × 10⁻⁴ and 2 × 10⁻⁴ respectively. As can be seen from Table 1 (C58-C1 (Ti-B6S3)) and Fig. 1, these transconjugant strains have acquired only one of the two plasmids present in the B6S3 donor strain. Indeed, the *EcoRI* digest fingerprint of the plasmid DNA in these transconjugant strains is identical to the *EcoRI* fingerprint of the plasmid DNAs from strain ACH-5 and from the transconjugants obtained with ACH-5 as donor. Furthermore, the fingerprint of the plasmid DNA from strain B6S3 contains as

well as all the bands found in the plasmid of strain ACH-5 a number of extra bands. These extra bands are supposed to be derived from the second, so called x-plasmid in strain B6S3. Strain B6S3 must therefore harbour two different plasmids, only one of which is necessary to convey oncogenicity and the capacity to catabolise octopine to a recipient *Agrobacterium*. This plasmid has an identical *EcoRI* fingerprint to the single plasmid present in strain ACH-5.

When the filters with the mixture of donors and recipients were incubated for 1 and 7 d on YEB agar, octopine-utilising recipients were detected with frequencies of 2 × 10⁻⁴ and 3.8 × 10⁻⁶, respectively. These colonies have been called C58-C1 *oct-u* (B6S3). As can be seen from Table 1, these are recipient strains that can grow on octopine and not on nopaline but they do not contain closed circular plasmid DNA nor can they induce tumours. Various methods¹⁴ were used to look for plasmid DNA, and also for plasmids either smaller or larger than the donor plasmid; none was found. When used as donors in similar crosses with C58-C1 recipients resistant to 200 µg ml⁻¹ streptomycin and 100 µg ml⁻¹ spectinomycin these colonies were unable to transfer the octopine utilisation marker. It

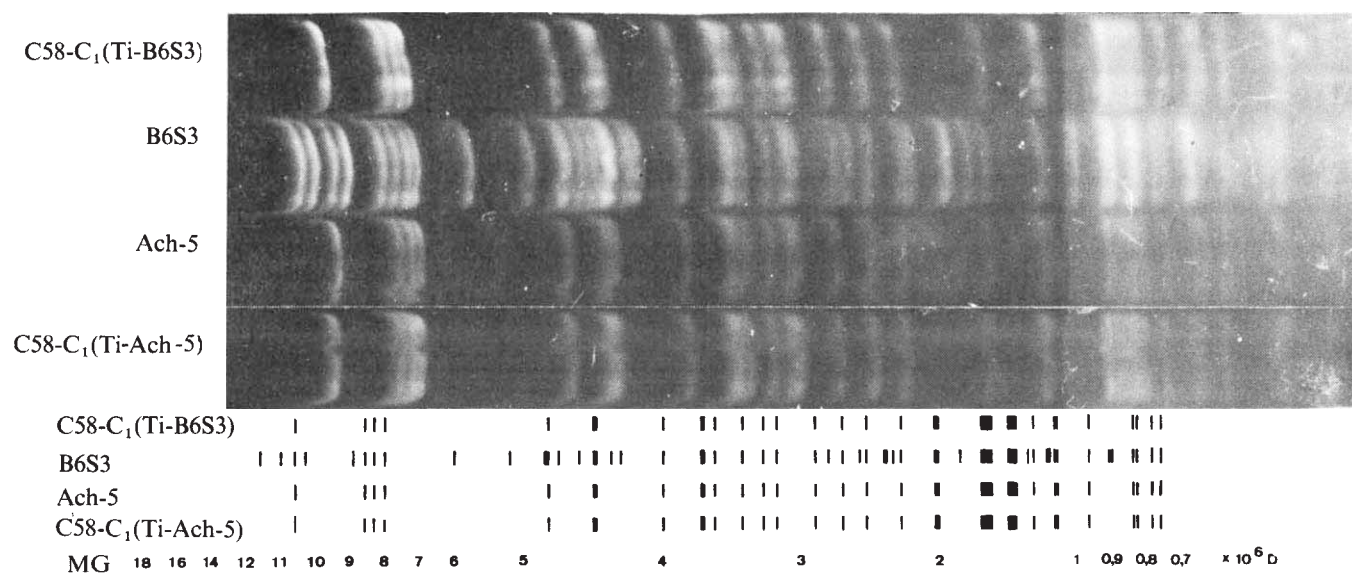


Fig. 1 Fingerprints of *EcoRI* digests of DNA from plasmids of donor and conjugant strains. The *EcoRI* enzyme was prepared according to Greene *et al.*¹⁹. Plasmid DNA of the indicated *A. tumefaciens* strains was purified on CsCl-ethidium bromide gradients, digested with *EcoRI* endonuclease and subjected to electrophoresis on 0.8% Agarose for 15 h at 2 V cm⁻¹ in the presence of ethidium bromide. The size of each fragment class was determined using *EcoRI*-treated λ DNA as the standard.

seems therefore that these strains have not acquired the whole Ti plasmid from the donor strain. The fact that such non-oncogenic, plasmidless octopine-utilising exconjugants could only be observed with strain B6S3 and not with strain ACH-5 as donor, could indicate that we are dealing here with a phenomenon similar to transduction. Indeed strain B6S3 is known to be lysogenic^{13,15}, whereas strain ACH-5 is not lysogenic. The main interest of these strains is that they indicate that the Ti plasmid genes that control octopine metabolism could be integrated into the chromosome of *Agrobacterium*.

A third cross was designed to test whether plasmids from nopaline-utilising strains also showed conjugative activity. As a donor strain C58 was used. This strain can degrade nopaline but not octopine and induces tumours that contain nopaline. It harbours a single plasmid 61.8 μ m long. As a recipient we used again the C58-Cl *rif-r ery-r* strain. As in the first cross, nopaline-utilising recipients were observed when the filters were incubated on a medium containing nopaline but not when the filters were incubated on YEB agar. The frequency of occurrence of transconjugants was much lower, however, compared with those observed with octopine-utilising donors. Indeed, incubation of the filters on nopaline-containing medium for 1, 4 and 7 d yielded transconjugants with frequencies of 10^{-7} , 10^{-5} and 10^{-4} respectively. As can be seen from Table 1, these transconjugants are oncogenic and have acquired the complete Ti plasmid from the donor strain. The observations described above confirm and extend those described in the companion paper by Kerr *et al.*¹⁰, and demonstrate that the Ti plasmids which determine oncogenicity in *Agrobacterium* strains are conjugative plasmids since transfer of intact Ti plasmids was observed by *in vitro* conjugation experiments. The possibility that the presence of the unusual, crown-gall specific, amino acids octopine and nopaline derepresses the genes involved in this transfer, must be considered seriously and would certainly explain Kerr's observations of *in planta* transfers^{8,9}.

An alternative explanation would be that the incubation on octopine agar in fact selects for rare exconjugants, increasing their number per recipient. This explanation, however, is unlikely, first, because rare exconjugants were not observed on media lacking octopine or nopaline (level of resolution better than 10^{-8}) and second, the doubling time of octopine-utilising exconjugants incubated on filters on octopine agar is about 3 h. The number of observed exconjugants after 24-h incubation of the filters on octopine agar (see also ref. 10) cannot therefore be explained by enrichment alone. The question therefore arises of whether a mechanism of induced plasmid transfer is involved in the transfer of plasmid-borne genes from the infecting bacteria to the plant cells.

We thank Drs A. Kerr and J. Tempé for discussion of the suggestion that octopine or nopaline is essential for Ti plasmid transfer. This work was supported by grants from the Kankerfonds van de ASLK and from the Fonds voor Kollektief Fundamenteel Onderzoek to J.S. and M.V.M. M.H. is a Navorsingsstagiair of the National Fonds voor Wetenschappelijk Onderzoek. A.D.P. is indebted to the Belgian IWONL for a fellowship.

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Tissue enrichments and protein turnover measured with ¹⁵N-glycine

VALUES for total body protein turnover (TBPT) measured in man using ¹⁵N-glycine^{1–3} are comparable with those obtained using ¹⁴C-amino acids⁴. Because ¹⁴C is radioactive it is of limited clinical use. The stable isotope ¹⁵N has a potentially wider application, although its use has been restricted for technical and conceptual reasons⁵. When a tracer labelled with ¹⁵N is used to measure TBPT the results are based on the enrichment of urea. This assumes that both urea and newly synthesised protein are derived from the same precursor N pool. Because urea is synthesised in the liver the enrichment of the precursor N pool for protein synthesis in the liver may determine the enrichment of urea. Thus the measurement of TBPT using ¹⁵N-glycine may be weighted heavily by the rate of hepatic protein turnover^{1,3}. Similar problems may arise when urinary ammonia is the end product (M.H.N.G. and J. C. Waterlow, to be published). The extent of labelling of the precursor N pool is affected by at least two factors. First, after infusion of a ¹⁴C-amino acid, its specific radioactivity at plateau is lower in tissues than in plasma^{6–9}, due to dilution with unlabelled amino acid derived from intracellular protein breakdown. This is termed internal recycling and also applies when ¹⁵N-glycine is infused. Second, when ¹⁵N-glycine is infused, extensive rapid intracellular transamination occurs especially in liver and kidney, so that the ¹⁵N is distributed to other amino acids, which will have a higher enrichment within the cell than in the plasma. Thus, depending on the balance between internal recycling and transamination, the intracellular enrichment of amino acids may be greater or less than the plasma enrichment. To investigate this problem we have infused ¹⁵N-glycine, and measured the enrichment of α -amino-N urea-N and

Table 1 Enrichments of N compounds in rat tissue after constant infusion of ¹⁵N-glycine

Tissue	α Amino-N	Urea-N (atoms % excess)	Ammonia-N
Whole blood	0.172 $\pm$ 0.017	0.118 $\pm$ 0.006	0.073 $\pm$ 0.011
Liver	0.296 $\pm$ 0.019	0.132 $\pm$ 0.004	0.134 $\pm$ 0.004
Kidney	0.224 $\pm$ 0.014	0.136 $\pm$ 0.003	0.129 $\pm$ 0.004
Muscle	0.170 $\pm$ 0.012	0.105 $\pm$ 0.006	0.080 $\pm$ 0.008
Brain	0.026 $\pm$ 0.003	—	0.076 $\pm$ 0.002

Values are means $\pm$ s.e.m.

Table 2 Total body protein turnover measured using different isotopes and end products

Isotope	Tissue sampled	Total body protein turnover (g kg ⁻¹ d ⁻¹)
¹⁵ N-glycine	Liver urea	36.3 ± 1.9
¹⁵ N-glycine	Kidney ammonia	36.4 ± 1.7
¹⁴ C-(U)-tyrosine*	Plasma tyrosine	35.0 ± 1.7 and 39.9 ± 2.6
¹⁴ C-(U)-tyrosine†	Plasma tyrosine	Between 32.1 ± 2.5 and 41.7 ± 9.6
¹⁴ C-(U)-lysine‡	Plasma lysine	30.7 ± 1.6

Values are mean ± s.e.m. Protein is assumed to contain 13% nitrogen and 3.12% tyrosine.

*Data from ref. 8.

†Data from ref. 12.

‡Data from ref. 7.

ammonia-N in various tissues. The TBPT calculated from the liver urea-N enrichment gives a value which we consider representative and which agrees with published values based on ¹⁴C.

Male albino rats weighing about 390 g were infused through a tail vein with a solution of ¹⁵N-glycine (97 atoms % excess, Prochem BOC, London) at a rate of 0.1239 mg ¹⁵N per h for 6 h. The rats were then decapitated and whole blood was collected in cold 10% (w/v) trichloroacetic acid (TCA). The liver, kidneys, quadriceps muscle and brain were homogenised in cold TCA. After centrifugation the supernatants were extracted twice with ether and alkalised with sodium hydroxide in a Leurquin apparatus¹⁰; the ammonia produced was collected in 0.1 N sulphuric acid by aeration. Urea in the NH₃-free extracts was converted to ammonia by urease (Type III Sigma) at pH 5.6 and collected by aeration. Amino-N was extracted from the ammonia-free and urea-free extract by the method of McFadyen¹¹. N-enrichment was measured in a double collector mass spectrometer (602 VG Micromass) after reaction of the ammonium sulphate with sodium hypobromite in a vacuum. TBPT was calculated according to the method of Picou and Taylor-Roberts¹. Significance was tested by the paired *t*-test.

Table 1 shows the enrichment of the three nitrogen compounds in the various tissues. The hepatic α -amino-N enrichment was nearly twice that in whole blood. The enrichment in renal α amino-N was significantly less than that in liver and greater than that in whole blood. This indicates that transamination was extensive in these organs and was a more important determinant of α -amino-N enrichment than internal recycling. The enrichment of α -amino-N was very much lower in brain than in other tissues studied. This is possibly due to a low rate of uptake of glycine by the brain with extensive internal recycling. Enrichments of α amino-N in muscle and whole blood were the same. There was no significant difference between urea enrichments in liver and kidney. Urea is first synthesised in the liver and then mixes with urea in other tissue pools, so urea samples isolated simultaneously from liver and kidney are originally synthesised at two different times. Since hepatic and renal urea-N have the same enrichment an isotopic steady state must have been achieved between these two pools. The lower enrichment of urea-N in muscle suggests that the muscle pool has not yet reached isotopic equilibrium with the liver pool at 6 h. Unequal enrichment of plasma and erythrocyte urea-N may explain why the enrichment in whole blood was lower than that in the liver and kidney.

There was no significant difference between liver and kidney ammonia-N enrichment or between ammonia-N and urea-N enrichment in these two tissues. Therefore TBPT can be calculated for each rat from the hepatic urea-N and renal ammonia-N data. The results show that there is no significant difference between values for TBPT based on the two excretory products. Furthermore, these results agree closely with those obtained after infusion with

¹⁴C-labelled amino acids (Table 2). This substantiates the validity of both methods and suggests that the highly enriched α amino-N in liver, which was not reflected in hepatic urea-N values, does not unduly influence TBPT results calculated from urea-N data.

The total α amino-N of the body does not form a single homogeneous pool. After an infusion of ¹⁵N-glycine the ¹⁵N label will be distributed to amino acids in a complex way depending on the size of the pools, their varied metabolism and differing turnover rates. Thus it is difficult to interpret or predict the enrichment of total α amino-N in any tissue. Nevertheless, the extremely high enrichment of hepatic α amino-N and the less highly enriched α amino-N in muscle, kidney and blood raises the question of the origin of urea-N. The data obtained show that none of the tissue pools of α amino-N can be identified as the single precursor for urea synthesis. The method of measuring TBPT, however, does not make any assumptions about the precursor enrichment for either urea or protein and does not require this enrichment to be measured. The method requires only that the nitrogen originating from glycine contributes to both protein and urea in the same ratio as the total α amino-N contributes to protein or urea. This assumption is supported by the close agreement between results obtained by ¹⁴C and ¹⁵N-glycine measurements, and between ¹⁵N-glycine and ¹⁵N-egg protein measurements².

If urinary ammonia-N enrichment is shown to be the same as renal ammonia-N enrichment, then urinary ammonia-N enrichment could be used to estimate TBPT in man. This would shorten the time required to reach plateau considerably and also simplify sample analysis.

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reviews

Functional biology of the mollusca

R. D. Purchon

Living Marine Molluscs. By C. M. Yonge and T. E. Thompson. Pp. 288. (Collins: London, 1976.) £6.00.

BEAUTIFULLY illustrated with sixteen plates (eight in colour) and with 162 informative line diagrams, this inexpensive book fills a major gap in libraries. It will be invaluable to university students; malacologists and palaeontologists professionally concerned with understanding functional morphology and the principles of molluscan evolution; conchologists; and enthusiastic amateur shell collectors.

The text, arranged in eighteen chapters all of a convenient size, is an easily understood narrative reviewing the functional biology and evolution of all the more important molluscan taxa, on a global scale. There is also a wealth of minor detail regarding the species of British marine mollusca.

After a brief introduction, chapter 1 is devoted to an historical review of molluscan studies to the present day. It remains for the reviewer to emphasise that the past half century has seen an explosive growth in the study of the functional biology of molluscs, to which no-one has contributed as extensively as has the senior author of this book.

Chapters 2, 3 and 4 deal with basic features of molluscan organisation and classification, and with the chitons which, with their eight overlapping shell plates and muscular foot, are adapted for movement over irregular hard surfaces. Chapters 5–9 concern the Prosobranchia, dealing successively with "The first gastropods": "Limpets and top shells"; "Mesogastropods" (which require two chapters); and with "Neogastropods". Here is a wealth of well-explained functional biology and ecological detail. These chapters portray the evolutionary plasticity of the Prosobranchia, and the resourcefulness of the Mesogastropoda in particular.

The next two chapters concern "Opisthobranch sea snails", and "Sea slugs". Reduction of the shell here leads to alternative chemical and biological protection against predators. After lightening, or loss, of the shell several lineages have independently acquired the ability to swim, the better to escape from their enemies. We can appreciate something of the grace and



Title page of first book devoted entirely to molluscs

beauty of these delightful animals from the excellent line and stipple drawings here.

Five and a half chapters are assigned to the Bivalvia, beginning with an account of the origin of primitive bivalves from a generalised molluscan ancestry, and covering the general organisation and mode of growth of bivalves. In "Evolution and adaptation of bivalves" we see a transition from deposit-feeding protobranchs to suspension-feeding lamellibranchs, with elaboration of ctenidial ciliary sorting mechanisms. These lamellibranchs exploit every possible aquatic habitat and mode of life, as is shown in chapters 14–16: byssal attachment to hard surfaces, with the adoption of heteromyarian or monomyarian form; swimming; shallow or deep burrowing in sediment, with a return to deposit feeding in the Tellinacea; commensalism; and boring in rock and timber by pholads and shipworms.

Chapter 17, "Anomalous bivalves and scaphopods", deals first with the Anomalodesmata, which lack hinge teeth and in some of which there is a lithodesma. The Verticordiidae indicate a transition from filter feeding to a scavenging or carnivorous habit, as also in the related Septibranchia. This was a necessary pre-adaptation towards life in abyssal sediments. Mention is made of the Watering Can Shell, *Penicillus*, which calls for a special effort by functional morphologists, who happen to be well-placed in the Indo-Pacific Region, to elucidate the

general biology and growth processes of early stages of this puzzling bivalve.

The Scaphopoda are shown to be either microphagous (*Dentalium conspicuum*), or carnivorous (*D. entalis*).

Chapter 18, "Cuttlefish, squid and octopus", relates the mantle cavity of these actively swimming molluscs to those of other molluscan classes. Attention is given to newly gained knowledge of the biology of the Pearly Nautilus, which moves slowly in moderately deep waters. Transition from ciliary to muscular ventilation of the mantle cavity permitted fast swimming, and required a high metabolic rate. This permitted the exploitation by cephalopods of waters from just above the seabed to the surface of the sea. In such pelagic hunters the statocysts, eyes, and brain are well developed, as also the tactile sense in octopods, which have secondarily returned to the seabed. A brief account is given of learning and memory in the octopus, and how knowledge of this has been gained by simple experiments.

The substance of the book is surveyed in an Epilogue. The mollusca arose with the contrasted advantages accruing from a bilaterally symmetrical body and a radially symmetrical protective shell; these, with the versatile radular apparatus, provided a basis for diversification to suit widely different habitats and modes of life. By acquisition of neutral buoyancy and muscular jet propulsion the cephalopoda outstripped all other invertebrates and now compete on equal terms with the marine (but not freshwater) Teleostei.

Erato voluta (Fig. 34, p86), until recently regarded as a cypreaeid, should be assigned to the family Eratoidae together with the genus *Trivia*. *Xylophaga* (p218) is reported as being unable to swallow fragments of the wood through which it bores, but this is not so; it has a moderately large caecum which is normally packed with wood fragments.

There is a useful list of recommended books for further reading, and there is a thorough index. Typographical errors are rare. □

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Chromosome studies in cancer cells

Cytogenetic Aspects of Malignant Transformation. (Experimental Biology and Medicine: Monographs on Interdisciplinary Topics, Vol. 6.) By N. B. Atkin. Pp. iii+168. (S. Karger: Basel, London and New York, 1976.) SFr/DM74.

THIS book is a critical review of the present state of chromosome studies in cancer cells by an author who has worked on these problems for very many years. Essential concepts relating to chromosome changes in malignant transformation are old and represent views which are now 'rediscovered' with more precise tools. In his evaluation of new evidence, the author draws on substantial material of his own, and the book therefore gains a particular authority. Such studies are central to the cancer problem, more so than many erudite and sophisticated lines of research, intellectually more favoured by grant-giving authorities. The new evidence clearly bears out that chromosome changes are non-random, clustering on particular

chromosomes; but many patterns are not yet discernible at histological and biochemical level, although such distinction must be present.

Some expected conclusions are not drawn by the author. The monoclonal nature of neoplasms sets a low limit to the number of cells capable of forming a clone in a tissue, the target cells for transformation. Other general principles seem to emerge. With a primitive stem cell transformation comes tumour-specific numerical chromosome imbalance, with a more derived stem cell transformation, single gene effects, that is, balanced translocations, and low numerical variation.

It may be that transformation is a process by which a clonogenic cell takes a retrogressive step in its phylogenetic pathway, thereby gaining a proliferative advantage over other cells. Further transformation often occurs within the clone. This study of 'embryogenesis in reverse' represents a formidable task, not likely to be quickly solved unless supporting disciplines work with these ideas in mind.

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Membrane separation

Membrane Separation Processes. Edited by P. Meares. Pp. ix+600. (Elsevier Scientific: Amsterdam, Oxford and New York, 1976.) Dfl. 250; \$96.25.

MEMBRANE separation processes, despite a long history, can only recently be said to have reached a stage of development where large scale practical applications are possible. At the same time, as a result of very many basic researches, the physics and chemistry of membrane processes have been clarified and a general formulation in terms of irreversible thermodynamics has been developed. It is therefore timely that a monograph on the theory and practice of membrane science should have appeared, in which actual and potential applications have been particularly emphasised.

The book is a cooperative effort to which well-known specialists in various technically significant areas have contributed. Such texts have both advantages and disadvantages—expertise in every topic covered in some, but some omissions, overlap and unevenness in others. This presentation, under the editorship of Professor Meares, has had considerable success in minimising the disadvantages, and the contributors

have ensured a high standard in the areas chosen.

The coverage can best be seen from the following chapter headings: the physical chemistry of transport and separation by membranes; liquid permeation through polymers; ultrafiltration; reverse osmosis in desalination; hollow fibres in reverse osmosis, dialysis and ultrafiltration; electro-dialysis; piezodialysis; gas separation by selective permeation; hydrocarbon separation by liquid membrane processes; enzyme membranes; separators and membranes in electrochemical power sources; ion-selective membrane electrodes; treatment of aqueous wastes and foods by membrane processes; and biochemical applications of membranes.

The book is well produced despite some printing errors, such as a mix-up in the middle of p201 and a misspelling of "repetitive" in the diagram on p94 and elsewhere. The contributors and editor are to be congratulated on a useful and comprehensive task well completed. The price of this book has unfortunately been set so high that many potential purchasers may be deterred and some libraries will also need to consider whether their funds permit them to buy it.

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Cyano complexes of transition metals

The Chemistry of Cyano Complexes of the Transition Metals. (Organometallic Chemistry: A Series of Monographs.) By A. G. Sharpe. Pp. xi+402. (Academic: London and New York, 1976.) £10.40; \$26.25.

THE publication of a comprehensive and up-to-date review of the cyano complexes of the transition metals is particularly timely. There is currently a rapidly growing interest in a whole new range of the related organic isocyano metal complexes, and it is appropriate that this volume indeed appears as a member of the excellent series of monographs on organometallic chemistry, edited by Maitlis, Stone and West.

Although the study of the metal cyano complexes goes back to the early eighteenth century, they continue to be a source of interest and research. Further, they present some of the most elegant examples for the teaching of the main principles of the structure and theory of the coordination complexes of the transition metals.

An introductory chapter deals with the general aspects of the cyanide ion along with the relevant structural, spectroscopic, thermodynamic and kinetic background. The rest of the book deals essentially with factual knowledge of the cyano complexes by individual groups in the Periodic Table, from the lanthanides to the copper and zinc groups.

The past fifteen years have seen a considerable growth in the literature of these complexes, and the author has surveyed this very extensive area with remarkable thoroughness. He does not, however, hesitate to draw attention to scientific results that he regards as either inconsistent or possibly unreliable.

The book contains over 1,800 references to the chemical and physical literature, but only a very brief subject index. Each chapter is, however, so consistently organised that I found little difficulty in finding what information, if any, was available about any particular cyano metal complex system.

Most organometallic and inorganic chemists will want this book on their shelves, not only as a valuable source of reference, but also as a pointer to many intriguing potential research areas.

E. W. Abel

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Synaptic transmitter substances

Neurotransmitter Amino Acids. By Neil Davidson. Pp. viii+179. (Academic: London and New York, 1976.) £5; \$12.75.

IN the past ten years there has been a rapidly growing interest in certain amino acids such as GABA, glycine and glutamate which are now generally believed to be important synaptic transmitter substances in the vertebrate central nervous system. Neil Davidson has undertaken the formidable task of writing a short book in which he aims to cover all aspects of neurobiology relevant to these amino acid transmitters.

The three opening chapters, which together occupy just over two-thirds of the book, provide an excellent account of the evidence that glutamate and aspartate, glycine, and GABA, are central transmitters. The references are usually well chosen but it is surprising that there is no mention of *N*-methobicycluculline or the immunohistochemical localisation of glutamate decarboxylase (GAD). The latter omission is particularly surprising since the localisation of GAD is potentially a valuable method of identifying GABA-operated synapses.

The author is at his critical best when discussing problems of electrophysiology and particularly the interpretation of experiments in which drugs are applied to neurones by iontophoresis. More neurochemical problems involving subcellular distribution, autoradiography and uptake, however, are not so critically assessed. For example, the author suggests that the presence of a high affinity uptake mechanism is a criteria for an amino acid transmitter, a view against which there is now much evidence.

There is an adequate chapter on taurine but the account of amino acids in retinal neurotransmission is rather disappointing. The role of GABA in the retina is discussed but taurine, glutamate and aspartate are barely mentioned, although there is much inferential evidence that they may be retinal transmitter substances.

The last two chapters provide an excellent account of the possible role of amino acids in presynaptic inhibition and a review of the behavioural and clinical correlations in studies of the neurotransmitter amino acids.

The book has a rather large number of typographical errors but of more consequence is the use of a bewildering number of units. For example, the concentrations of amino acids are expressed in at least five ways making

ready comparisons difficult. It would be helpful if in future editions the author would recalculate amino acid levels in one unit such as $\mu\text{mol g}^{-1}$. These are, however, minor criticisms, and the author is to be congratulated on producing a very readable introduction to amino acid transmitters. This book will be of great value to both undergraduate and postgraduate students, and I suspect that many involved in research on amino acid transmitters will keep a copy of this book readily available.

M. J. Neal

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Gerontological observations

The Ageing of Connective Tissue. By David A. Hall. Pp. vi+204. (Academic: London and New York, 1976.) £4.80; \$10.50.

OVER the past decade considerable advances have been made in our understanding of the biosynthesis and structure of the components of connective tissue. The deterioration of connective tissue is an obvious fact of ageing, and connective tissue biochemists should be encouraged to apply this new fundamental knowledge and accelerate the emergence of gerontology from its classical descriptive phase. Certainly it is becoming increasingly obvious that any breakthrough in the understanding of ageing can only come from studies at the molecular level. Dr Hall has therefore chosen his time well to write his monograph.

The book is well organised to provide the reader firstly with a brief resumé of the current theories of ageing and then with an overall view of the breadth of involvement of connective tissue components in various tissues, starting at the macroscopic level and working gradually down to the complex molecular level.

The book does, however, contain a number of errors, some obvious and some not so: for example, the gal-glc disaccharide is stated as being attached to hydroxyproline instead of hydroxyllysine; the statement that $(\alpha_2)_3$ exists in tissues; the stated presence of hydroxylisnonorleucine in elastin; and a number of incorrect cross-link formulae. There are also a number of surprises: for example, quotation of the old, unconfirmed observation that cellulose is present in older human skin; the electron micrograph picture of metacollagen (thermally denatured skin collagen); the considerable over-emphasis on pseudo-elastin (an unfortunate

name, since it is surely no more than highly cross-linked impure collagen). The statement that elastase, a serine protease similar to chymotrypsin, has no effect on collagen is incorrect, a fact which throws some doubts on the conclusions drawn from these experiments.

I did not, therefore, feel that the promise of the first half of the book was fulfilled when the biochemical basis of the age changes was discussed. More importantly the emphasis in this part of the book on old unconfirmed and often confusing work, together with the numerous errors of fact that have been overlooked, mean that the book has to be read with considerable wariness. This is particularly important since the stage is now set—and Dr Hall has played no small part in this—for some critical studies on the role of connective tissue in the ageing process. Despite these reservations, it is hoped that this compact and inexpensive book will engender the right kind of enthusiasm for establishing the biochemical and molecular basis for gerontological observations.

Allen J. Bailey

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Multitude of fishes

Fishes of the World. By J. S. Nelson. Pp. xiii+416. (Wiley-Interscience: New York and London, October 1976.) \$31.85; £18.50.

MODERN estimates of fish abundance have reached 30,000 species, although the present author arrives at a conservative and precise 18,818. Nowadays, to encompass the fishes even at family level is a daunting task, further complicated by much revision of their higher classification within the past fifteen years.

The purpose of this book is "to present a modern introductory systematic treatment of all major fish groups", the basic units being all extant, and selected extinct, families of fishes, from jawless ostracoderms to choanate crossopterygians. As a preliminary, protochordates and even conodonts are also discussed. The family category is, of course, a subjective taxon, but the author is not extravagant by previous standards in recognising 450 of these in 46 orders of true fishes. Overall classification follows accepted recent schemes, although more radical alternatives are mentioned. Each family receives a common name, a concise statement of distribution, essential diagnostic features, sometimes further division into subfamilies, and biological notes when of better known status. A line drawing of a typical species is provided for most families. Appendices contain a simplified classification and a series of 45 world distribution maps. A useful bibliography of about 600 references (though none more recent than 1973) and a systematic index, complete the work.

This is a pleasing book, not only in much of its content but also in typography, format and illustration. Factual coverage for each family is necessarily brief, but random consultation proved consistently successful even for such diverse forms as *Schindleria* (the lightest vertebrate), parasitic eels (Simenchelyidae), the dreaded candiru (Trichomycteridae), and Baikal oilfishes (Comephoridae). Treatment of the gobioid fishes—representing perhaps one-tenth of all living teleosts—seems restrained and practicable in comparison with more frenetic and provisional specialist schemes.

Among shortcomings, fossil groups receive uneven and often perfunctory attention, out of keeping with current efforts to integrate classification over the entire time range of fishes. A work for general reference also needs a synopsis of fish anatomy, especially

osteology, to explain diagnostic characters, perhaps replacing much of the rather generalised introduction and the 24 pages devoted to maps. Use of recognised terminology for faunal complexes could have given a more precise and biologically significant statement of family distribution. Finally, comparison with a recent Russian work by Lindberg, also entitled in translation *Fishes of the World*, and involving the same publishers, emphasises the absence of a key to families from Nelson's account. Keys are not only altruistic but also cathartic against taxonomic subdivision. In a number of ways, by accident or design, the two monographs are unfortunately complementary rather than competitive and it is a pity that a little more on bones, fossils, and identification could not have been included between the relatively expensive covers of the one now reviewed.

P. J. Miller

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Radioimmunoassay

Radioimmunoassay of Biologically Active Compounds. By C. W. Parker. Pp. xiv+239. (Prentice-Hall: Englewood Cliffs, New Jersey, 1976.)

RADIOIMMUNASSAY is a technique the possibilities for which were realised, at least partly, by endocrinologists a good many years ago. They are, however, far wider than is even now recognised. In principle any soluble substance with which a specific antibody can react is capable of being estimated at the nanogram level or better, even in the presence of large quantities of nonspecific material. At one extreme, therefore, proteins, which are intrinsically immunogenic, and at the other simple chemicals, such as drugs which can be stably attached to 'carrier' proteins and made immunogenic, are open to estimation in grossly heterogeneous mixtures, such as serum and crude tissue extracts. The intermediate group, the medium sized polypeptides of about six to about fifty residues—a group which includes many important hormones—is in fact the trickiest problem. But essentially the problem boils down to good chemistry, so as to attach one's substance undamaged and the right way round; and competent immunology, so as to raise antibodies of—and this is not always so easy—satisfactorily high affinity: which just means that the antigen-antibody complexes must remain bound even at very high dilution. The sensitivity of the

test—that is, the smallest concentration per millilitre which can be quantitated—is directly dependent on the affinity attained.

The great strength of this book, which can be recommended without reservation, is that Dr. Parker has realised that the principles and practice of immunogen preparation and of immunisation are in importance equal to or greater than, the details of the actual tests, so that the former are considered at length and with invariable common sense. He treats clearly, thoroughly and from first principles problems of affinity and of cross-reactivity, and of factors in tissue extracts or serum such as degradative enzymes, which may affect assays. He also refers to methods alternative to the standard ones—for example when dealing with the separation of free from antibody-bound antigen, he considers chromatographic or molecular sieve separation as well as the more conventional salt-precipitation or double-antibody techniques.

There are two techniques related to radio-immunoassay which may be of increasing importance in the future, to which some but no very extensive reference is made. These are the use of endogenous blood or cell receptor proteins instead of antibody, and the method known affectionately as Eliza, the enzyme-linked immunoassay. About the first, the general conclusion is that it is usually easier to raise an antibody than to purify a specific receptor protein: I would suggest, however, that in some cases—using, for example, suspensions of cultured cells or blood leukocytes—a highly sensitive assay might be contrived for quite small radiolabelled substances, which might be difficult by any other method. As far as Eliza goes, since this involves an antibody, practically everything in this book is as applicable to it as it is to a radioimmunoassay. The great advantage claimed for Eliza is that it is not dependent on a large and expensive counter which is always breaking down, but on a simple colorimeter. The whole question is whether an enzyme assay ending in a coloured product (generally using peroxidase or alkaline phosphatase) can be made as sensitive as a count of radioactivity. The evidence is, rather surprisingly, that under favourable circumstances it can.

This book is the most intelligent one which I have met for developing and advancing the subject; it cannot fail to be of continuing importance in biology and medicine.

P. G. H. Gell

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Free radicals in biological systems

Free Radicals in Biology. Vol. 1: Pp. xv+287. Vol. 2: Pp. xiv+303. Edited by W. A. Pryor. (Academic; New York and London, 1976.) £19.55; \$27.50 each volume.

PUBLICATION of a series of volumes devoted to the role of free radicals in biological systems is certain to be of interest to those workers who are particularly concerned with the relationship between events at the basic chemical level and their biological consequences. The first two volumes of such a series have appeared under the editorship of W. A. Pryor, the stated aim being to present reviews which, for specialist and generalist alike, may be primary sources of reference as well as short up-to-date surveys. This aim is clearly satisfied in several of the contributions.

The first volume opens with a general survey of the chemical reactions of free radicals; written by the editor, it is also, to some extent, an introduction to the series. A short chapter by Mead presents the evidence for the involvement of free radicals in the autoxidation of lipid membranes both *in vitro* and *in vivo*. It is inevitable that the technique of electron spin resonance (ESR), so widely used for the detection and identification of free radicals, should be given a prominent place in the first volume. Basic concepts and characteristics of ESR spectra, together with some applications to biological free radicals, are covered by Borg. The technique of spin labelling (the introduction of paramagnetic probes) is very powerful in investigations of structure-function problems in biology and is discussed in some detail by Smith, Schreier-Muccillo and Marsh; I gained the impression, however, that this subject area is slightly out of context in this particular volume. One chapter is devoted to free radicals in photosynthesis (Loach and Hales), particular attention being paid to those involved in the primary events in bacterial photosynthesis. A very clear account of the function of the superoxide dismutases, catalases and peroxidases in the metabolism of O_2^- and H_2O_2 is given by Fridovich.

There is greater emphasis on chemistry in volume 2. Pyridinyl radicals, which are models for those derived from nicotinamide adenine dinucleotide (NAD), are discussed by E. M. Kosower, and an account of radical and radical-ion reactions in the glutathione-glutathione disulphide system is given by N. S. Kosower and E. M.

Kosower. There is a great deal of current interest in the production and reactions of singlet oxygen; it is appropriate, therefore, that a chapter (by Foote) deals with the intermediary function of this reactive species in photosensitised oxidations and in certain biological systems. The atmosphere is not neglected, and the mechanism of photochemical smog formation (Kerr, Calvert and Demerjian) and the toxicity of air pollutants such as nitrogen oxides and ozone (Menzel) and peracyl nitrates (Mudd) are discussed in terms of possible free radical intermediates. The controversial question of the presence of free radicals in dry biological systems is reviewed by Heckly who shows that many of the claims are due to arte-

facts. Henriksen *et al.* describe the effects of ionising radiations on dry protein, nucleic acids and related substances, particular emphasis being given to ESR studies of single crystals of the components of these macromolecules; the production of free radicals in such a manner will clearly be of great radiobiological significance.

These two volumes cover a fairly broad area and the series as a whole should therefore be valuable source material for those involved in the chemistry-biology interface.

George Scholes

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Concanavalin A

Concanavalin A as a Tool. Edited by H. Bittiger and H. P. Schnebli. Pp. xv+639. (Wiley-Interscience; London and New York, October 1976.) £19.50; \$38.50.

CONCAVALIN A has had a curious history. It is of course only one of a very large number of lectins, and was by no means the first one to be used extensively in biology. The father-figure of cell biology, Paul Ehrlich, for example, was using other lectins before 1900 in a whole series of beautifully designed experiments showing the interactions of lectins with cell surfaces. Concanavalin A was, however, first to be crystallised by Sumner in 1919, and a pure protein became available rather than crude extracts of seeds which were then (and also sometimes still are) used as a source of lectins. The amino acid sequence and three-dimensional structure of concanavalin A were determined by Edelman and his colleagues, and the specificity of carbohydrate binding was worked out in detail, largely by Goldstein and his colleagues. Since then concanavalin A has been used in an astonishing range of experiments and is now a standard laboratory reagent, both in solution and as an immobilised reagent for affinity chromatography. It is, therefore, not surprising to see a book dedicated to this most versatile reagent.

Concanavalin A as a Tool is, however, much more than a collection of data concerning one particular lectin. It is, in effect, both a source book and a laboratory aid to many of the techniques in routine use in cell biology laboratories. There are 61 chapters, each written by an expert or experts and ranging from isolation, structure and specificity, histochemistry (fluorescence microscopy and electron micro-

scopy) to separation methods using immobilised lectin, agglutination reactions, lymphocyte mitogenesis and a host of biological applications—for example, selection of lectin-resistant cell lines.

Each chapter is short and to the point (few are more than half-a-dozen pages) but each is self contained in giving just enough background to illustrate the particular application being described. The main feature of most chapters is a "Methods Section" which provides sufficient detail to be used in the laboratory. The list of useful methods is too long to mention here and presumably will vary in content depending on the user. Some typical examples are induction and titration of concanavalin A antibodies, preparation of conjugates (FITC, ferritin as well as con A-trypsin and con A-asparagine conjugates), labelling with radioiodine or tritium, agglutination assays (with excellent discussion of the many pitfalls), preparation and properties of divalent derivatives, binding assays and analysis of heterogeneity of receptor sites, and the preparation and use of affinity columns. An appendix lists commercially available reagents and derivatives.

In short, Bittiger and Schnebli must be well satisfied with their efforts and those of their contributors. *Concanavalin A as a Tool* is a book to own and keep close by the bench. It also makes good reading despite the large amount of information packed into a relatively small space and constant change of authors—an indication of some excellent editorial work. If the price seems substantial (although not excessive), the purchaser is really getting two books for the price of one.

R. C. Hughes

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Nucleon-nucleon interaction

Introduction to the Strong Interactions. By Nathan W. Dean. Pp. xiv+377 (Gordon and Breach: New York, London and Paris, 1976.) £19.70.

THE forces which bind the nucleons in a nucleus are called 'strong interactions', because their characteristic coupling constant g (the analogue of the electric charge e in electromagnetic interactions) satisfies $g^2/4\pi \approx 14.7$. Consequently, perturbation theory is useless in this area, and the applications of field theory to the subject have been far less fruitful than applications to weak or electromagnetic processes. Indeed, there has been a flourishing school of thought dedicated to developing the (S-matrix) theory of strong interactions without any reference to field theory. This book is not committed to such a position, but it does boast that it "is the first to unite under one cover introductory treatments of all three of the primary facets of the theory of strong interactions . . . without the unnecessary encumbrances of field theory." As such it has a rather old-fashioned slant, since modern developments have leant rather heavily on there being an underlying strong interaction field theory. But the approach does have the advantage that the material is immediately accessible to first-year graduate students.

The first of the three main topics covered is group theory. This is mainly SU(3), of course, which is approached by way of the rotation group, the isospin group, and the Sakata model. There is quite a lot of material here, including the Young tableaux for the representations of SU(N), but not the rules for the decomposition into irreducible representations of a general product of two such representations. Curiously, the Gell-Mann Okubo mass formula is not really derived. There is a chapter on (the old) SU(4), and SU(6) as applied to multiplets, but no coverage of SU(6)_w or the Mellosh transformation.

The second and third sections use potential theory to explore the analytic properties of the (non-relativistic) scattering amplitude, so as to motivate the assumed analytic structure of the relativistic amplitude. The former part is devoted to the study of the singularities in the complex energy plane, and includes dispersion relations for forward πN and NN scattering, the Mandelstam representation, and the N/D method. The final section deals with Regge theory, up to and including Regge cuts, duality and the Veneziano model. The treatment in both of these sections follows fairly well-worn tracks

and is clear and easy to follow; indeed, this is true of the book as a whole.

There is, of course, much that could have been included in such a book, as the author freely concedes. The restriction of coverage to two-body scattering, however, seems to be unnecessary and undesirable. Much of the recent work on inelastic processes (I am thinking of hadronic scaling) needs no field theory and can easily be understood by beginning graduate students. And these processes constitute the vast majority of high energy processes actually seen. So it really does not seem good enough to say simply that an understanding of these processes cannot "progress until two-particle scattering is well understood." After all, many of these students will be writing their theses on just such processes. I dare say that the author would want to distinguish between theory and phenomenology, but this is not the place to pursue that discussion.

As a whole the book is lucid and well written. The coverage is limited, as I have detailed. The word *Introduction* in the title means just that. The price is no more exorbitant than most books seem to be these days.

D. Bailin

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Higher mental processes

Language, Memory and Thought. (The Experimental Psychology Series.) By J. R. Anderson. Pp. xiii+546. (Lawrence Erlbaum Associates: Hillsdale, New Jersey, October, 1976. Distributed by Halsted Press, a Division of Wiley: New York and London.) \$26.50; £15.40.

IN this book, Anderson has attempted to bring together the artificial intelligence (AI) and psychological methodologies in order to devise a theory of higher mental processes. The theory has grown out of his dissatisfaction with a model of human memory that he developed with Gordon Bower.

The new model is called ACT and consists of two main components: a memory for facts, and a set of procedures for acts. The memory is an associative network in which facts are represented in an abstract 'propositional' form. Only a very restricted part of it is active at any one time, but this activation spreads through the network, subject to a periodic dampening of all but a few protected elements that function as a short term memory. These protected elements do not decay but are simply displaced by new items of information from time to time.

The procedures representing knowledge of how to do things constitute

what is known as a "production system": each "production" is a simple rule that tests for certain conditions in the active part of memory and, if they obtain, carries out a specific action on the contents of memory or the real world. The analogy with the old idea of a stimulus-response bond is deliberate. The way in which a production is selected and applied is a complicated affair: first, the relevance of all the productions in the system is tested in parallel, and the ones that match the contents of active memory applied.

Anderson spells out the complexities in detail and provides numerous examples of production systems that have been implemented in computer programs. He then explores some of the model's consequences for such matters as memory for sentences and prose, inferential mechanisms, the production and comprehension of language, and its native acquisition by children.

The author writes that he is essentially delivering an interim report of work in progress. He also argues in the first and most provocative chapter that it is impossible to identify uniquely the nature of mental processes. It may not, moreover, be possible to determine whether a particular sort of information is represented as a visual image or in some more abstract propositional form; or whether a certain mental process is carried out serially or in parallel; and so on. He seems to have reached this conclusion as a result of writing the book because, as he acknowledges, the model derives from his intuitions about precisely such matters.

Anderson is at pains to provide empirical evidence for ACT. Some of his experiments, however, are weak; none of them is so striking that it could not have been devised, and its results predicted, from an entirely different theoretical standpoint. An experiment on syllogistic inference, for example, relies on his own intuitive assessment of the difficulty of different syllogisms. He also elucidates the power of ACT by theoretical arguments of considerable technicality culled from automata theory and formal logic. Some of these arguments are rather irrelevant. It is not a great deal of use, for example, to give a precise formal specification of the semantic power of the model when it is obvious that it cannot handle modal notions like "possibility", and that it seems to have no way of distinguishing between facts and events. Despite the overall impression of great enthusiasm and energy, the book is not entirely convincing.

P. N. Johnson-Laird

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obituary

Charles Enrique Dent, Professor of Human Metabolism at University College Hospital Medical School (UCHMS), died on September 19, 1976. He was born in Spain in 1911 of a Spanish mother and English father but received his schooling in Bedford and Wimbledon College, London. He started work at 16 as a bank clerk and studied chemistry at evening classes. He won a scholarship to Imperial College, London, and in 1931 graduated with a first class honours degree in chemistry. He spent a further two years there making a study of copper phthalocyanin for his Ph.D. thesis and then worked for three years in the dyestuffs division of ICI in Manchester; many of the blue dyes in use today have their origins in his work at that time.

In 1937, at the age of 26, Dent started his medical education at University College, London, only to have it interrupted two years later by the outbreak of war. As a Territorial he was among the first who went to France with the British Expeditionary Force and was engaged in military intelligence. He was besieged in Arras, mentioned in dispatches and evacuated from Dunkirk. After a short spell back at medical studies he was called by the War Office to undertake counterespionage work in Bermuda; by applying his chemical knowledge to detect messages secreted in mail passing between the United States and Europe, he helped to uncover an important spy ring.

At 33, Dent at last had the chance to qualify MB, BS and he became the last house physician to the celebrated cardiologist and clinical scientist Sir Thomas Lewis. Soon after, he passed the Membership examination of the Royal College of Physicians and was appointed an assistant to the Medical Unit of UCHMS under Professor (later Sir Harold) Himsworth. He was promptly seconded to a Medical Research Council team which went to Belsen concentration camp to study the value of protein hydrolysates in the treatment of severe starvation. Subsequently he began his studies of amino acid disorders, making use of the technique of paper chromatography which had only just been described by Martin and Synge. He improved the technique and his paper on the behaviour of some 60 amino acids and other ninhydrin-reactants on phenol/collidine filter paper chromatograms, which was published in the *Biochemical Journal* in 1948, rightly became a classic reference because it

represented the culmination of a vast amount of meticulous work. He then proceeded to apply this tool to the study of various inborn errors of metabolism, helping to elucidate the precise defect in known amino acid disorders and discovering previously unrecognised syndromes. He contributed greatly to knowledge of renal tubular disorders and of mental defects caused by metabolic errors. In less than 10 years he became a world authority on this group of disorders, sparking off a new interest in them.

The study of renal disorders led on to an interest in metabolic bone disease and impressed by what he learnt when he visited Fuller Albright's department at the Massachusetts General Hospital while holding a Rockefeller Fellowship in 1946-47, he resolved to persuade UCH to build him a metabolic ward. This finally materialised in 1951 and from then on his research interests progressively shifted towards the study of calcium and phosphate metabolism. He systematically studied hyperparathyroidism, osteomalacia and the actions of Vitamin D, but he also acquired an unrivalled experience of rare bone diseases as patients and skeletal radiographs were referred to him from far and wide. He obtained the MD(Lond.) in 1949, became Reader in Medicine at UCHMS in 1951 and was elected Professor of Human Metabolism in 1956.

Despite Dent's insistence on a scientific approach to medicine, he remained essentially a clinician and his ward became a Mecca for doctors from all over the world. Many honours came his way, including honorary MD's from Louvain and Uppsala Universities, Fellowship of the Royal Society, the Gairdner Foundation Award and, in the 1976 New Year Honours, the CBE. In spite of his fame he remained modest, approachable, and greatly concerned for the welfare of his patients. He also put much time and effort into the teaching of medical students and for many years he was Staff President of the Student Union. He was very hard-working and his enquiring and original mind, tremendous enthusiasm and readiness to share ideas made him a stimulating colleague and an inspiring teacher. He contributed 166 scientific papers to the medical literature, many shared with the small research team he directed.

In spite of Dent's prodigious medical achievement he had the capacity to enjoy many other things. He was a keen squash player, made wine from

his own vines, reared trout, played the flute, and enjoyed entertaining in a relaxed and happy home. He was fluent in Spanish but was also able to lecture in French, Italian and Russian. His final illness emphasised his great stature for though aware of its relentless progress he remained cheerful and hard-working, sustained by his strong religious faith. He is survived by his wife, son and five daughters.

F. V. Flynn

Dr W. F. Bewley, CBE, DSc, VMH, died on December 11, 1976. In his death, agricultural research has lost one of the few remaining scientists who were concerned with the expansion of research facilities in the early 1900s which laid the foundation for the present Agricultural Research Service. Following the scheme initiated in 1911 by the then Board of Agriculture and funded by the Development Commission, Fleming Bewley joined the staff of the Rothamsted Experimental Station in 1912 as assistant bacteriologist to Dr H. B. Hutchinson in the James Mason Laboratory. In 1919 he was appointed mycologist at the Experimental and Research Station, Cheshunt, which had been established in 1914 and had close connections with Rothamsted. He was promoted to Director in 1921, a position he held for 34 years until the Station closed in 1955. With the formation of the Glasshouse Crops Research Institute in 1953 he became its first Director and was responsible for the initial development of the Littlehampton site, for the assimilation of the work of the Mushroom Research Association and for the transfer of the staff and facilities from Cheshunt to Littlehampton in 1955. Following his retirement in 1956 he took a keen interest in local government and was Chairman of the Worthing Rural District Council.

Early in his career he established his authority as a plant pathologist. His book *Diseases of Glasshouse Plants*, first published in 1923 was for many years a work of reference. He applied his mycological knowledge to the cultivation of edible fungi and his book *The Cultivation of Mushrooms* ran to three editions. In 1939 he had almost completed a comprehensive handbook *Commercial Glasshouse Crops* but publication was delayed to 1950 by the war. Though it became quickly outdated by rapid technological development in the 1950s it remains as a

monumental record of glasshouse crop husbandry in the first half of this century.

Fleming Bewley was a man of character and of strong will who was wholly dedicated to serving the glasshouse industry in the United Kingdom and in the Channel Islands. The industry owes him a great debt and he will be remembered not least by the developments he initiated in disease control, biological control of glasshouse pests, virus free seed and CO₂ enrichment.

G. F. Sheard

James Edgar Dandy died at his home at Tring, Hertfordshire, on November 10, 1976, leaving the ranks of taxonomic botanists sadly impoverished. He was born at Preston on September 24, 1903, and attended the Grammar School there until entering Downing College, Cambridge, where he graduated BA in the Natural Sciences Tripos in 1925. In that year he was appointed a temporary assistant in the herbarium at The Royal Botanic Gardens, Kew, where for two years he worked with the late Dr John Hutchinson, a taxonomist of deep understanding and wide experience who, at that time, particularly specialised in the African flora and in the genus *Rhododendron* but who was also formulating his ideas for a new system of classification of the Flowering Plants. Hutchinson's powerful yet genial influence greatly stimulated Dandy and his first publications in joint authorship with Hutchinson appeared in 1926. Again, prompted by Hutchinson's questing approach to classification, Dandy undertook an investigation of the *Magnoliae* (though he also worked on the families *Loasaceae* and *Saxifragaceae*) and he published a number of papers on the group though, alas, his most important contribution and indeed the culmination of his botanical life-work, a monograph of the genus *Magnolia* the result of nearly fifty years of research, remains in manuscript because of his reluctance to accept the work as sufficiently complete by his own exacting standards.

In 1927 Dandy was appointed Assistant Keeper in the Department of Botany, British Museum (Natural History) and during the war became responsible for the collections evacuated to Tring where he made his home. He became Keeper of Botany in 1956 and when he retired in 1966, relieved to shed his administrative load, he was able again to concentrate on his research. While Keeper he completed two important works, his *List of British Vascular Plants* (1958) and *The Sloane Herbarium* (1958). The first work is a bible for botanists who seek the correct name of any British flowering plant or

fern and it has been unquestionably accepted as the key work and a tribute to his unequalled knowledge of nomenclature and taxonomic insight. It was a fortunate circumstance that enabled the merger in Dandy's *List* of three similar previously independent and competing lists.

The Sloane Herbarium is a work in which Dandy deployed his discriminating scholarship and the full scope of his incisive and analytical mind. The main commentary to the *Sloane Herbarium*, which was acquired by the nation with Sir Hans Sloane's other vast collections in 1753 for £20,000 as the actual foundation of the British Museum, was in the form of loose paper slips on which Mr James Britten, a member of the staff for thirty eight years, had recorded the results of his scrutiny of the herbarium and its history. Members of the staff continued to add additional information and in 1953 it seemed appropriate in the bicentennial year of the British Museum, that this valuable commentary, of immense value to botanists throughout the world, should be published. Initially the task of editing was entrusted to Mr Spencer Savage, formerly Librarian and Secretary of the Linnean Society who was well versed in the history of botany at the time of Sloane and Linnaeus but to complete the work needed the critical understanding of an experienced taxonomist with a deep knowledge of the relevant literature. Dandy was persuaded to undertake this charge and he examined every sheet of the 265 copious volumes, carefully revised and edited all that had previously been written and produced a volume of the utmost importance.

Other valuable publications by Dandy are his *Index of Generic Names of Vascular Plants 1753-1774* (1952), the result of 25 years labour with about 3,000 entries each checked by reference to an extensive literature and his *Watsonian Vice-Counties of Great Britain* (1969), a basic work in recording the distribution of British Plants. Both these volumes embody years of painstaking investigation by one with a keen, well-ordered mind.

Dandy developed a great interest in aquatic Monocotyledons, especially in the families *Hydrocharitaceae*, *Najasaceae* and *Potamogetonaceae* and some of his conclusions were used by Hutchinson in *Families of Flowering Plants*. I was associated with Dandy as a colleague and firm friend for nearly fifty years and it was our intention eventually to prepare a monograph of the genus *Potamogeton* in Britain and though much preparatory work was done in a series of joint papers our careers so diverged that close collaboration became extremely difficult and

thus our aim was never achieved. Dandy, however, continued his studies in the group and was its undisputed authority and in other aquatic genera. For some years he had been unhappy about the identity of a plant only found in Britain in Esthwaite Water and accepted by botanists as a species of *Hydrilla* from the Baltic region. Detailed morphological study enabled Dandy to place the plant in the related genus *Elodea* and so established an additional amphiatlantic species, *E. nuttallii* as a member of the British flora.

His ungrudging help is acknowledged in countless publications and no one—and they were many—appealed for his assistance in vain. He was an inveterate perfectionist with high intellectual integrity, so much so that he left much of his material unpublished, and yet unsparingly scrutinised and polished the contributions of others. He was highly critical of sloppy writing in scientific papers and he did a great deal to ensure the highest possible standards in all matters referred to him. It was particularly in the legalistic realm of nomenclature in which he was an acknowledged international authority that Dandy's advice was constantly sought and willingly given. Many works including the prestigious *Flora Europea* bear the stamp of his influence.

Dandy was made an Honorary Fellow of the Royal Horticultural Society in recognition of his work on Magnolias, especially for help given with the publication of G. H. Johnstone's *Asiatic Magnolias in cultivation* which was sponsored by the Society. He was an Honorary Member of the Botanical Society of the British Isles of which for a period he was a Vice-President. He was elected to Fellowship of the Linnean Society in 1927. The genus *Dandya* was named in his honour by his friend Professor H. E. Moore of Cornell University.

Dandy in his younger days was a keen footballer and supporter of Preston United in their heyday. He played in the position of goalkeeper for his College and also in the staff team of the Natural History Museum. He thoroughly enjoyed his angling exploits and on several occasions he scaled the high defences of Gunnersbury Park before the place officially opened so that he could secure his favourite swim for carp. The lake at Blenheim with a particular tench-holding deep was another preferred spot. He was also an excellent bridge player.

This accomplished, modest, generous and earnest man will be much missed, especially in the botanical world.

In 1929 he married Joyce Isabelle Glaysher and they had a son and a daughter (deceased). **George Taylor**